ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume XLVIII, Art. 2. Pages 31-218

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ANTIBIOTICS

Parts I and II

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NEW YORK
Published by the Academy
September 27, 1946

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ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
VOLUME XLVIII, ART. 2. PAGES 31-218
SEPTEMBER 27, 1946

ANTIBIOTICS[*]

PART I: MICROBIOLOGICAL

PART II: PHARMACOLOGICAL

BY

WERNER W. DUEMLING, GARFIELD G. DUNCAN, WILLIAM H. FELDMAN, J. W. FOSTER, A. HERVEY, H. C. HINSHAW, M. J. JOHNSON, F. KAVANAGH, A. KLEINMAN, HANS MOLITOR, EDWIN PULASKI, GEOFFREY RAKE, KENNETH B. RAPER, D. M. REYNOLDS, ARTHUR P. RICHARDSON, W. J. ROBBINS, H. ROBINSON, A. SCHATZ, SELMAN A. WAKSMAN, AND H. B. WOODRUFF

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[*] This series of papers is the result of a Conference on Antibiotics held by the Section of Biology of The New York Academy of Sciences, January 17, 18, and 19, 1946.

[†] Publication made possible through a grant from the Conference Publications Revolving Fund.

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ANTIBIOTICS

PART I

MICROBIOLOGICAL

ANTIBIOTIC SUBSTANCES—CONTRIBUTION OF THE MICROBIOLOGIST*

By Selman A. Waksman

New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey

Just about five years ago, at the meetings of the Society of American Bacteriologists in Saint Louis, during Christmas 1940, the author was requested to preside over a round table discussion on the subject of the production of antibacterial substances by microorganisms. Both the organizer of that round table, Dr. Dubos, and the proposed Chairman failed to secure enough participants for that two-hour discussion. No better example can serve to illustrate the tremendous progress made in this new branch of science, namely that of Antibiotics, than to point to this Conference which we are about to open. It was, now, not a question what subjects to discuss and what speakers to invite, but to decide what could be left out and who among the numerous workers on this subject were most able to represent the various aspects of this field of research and application. Certain important aspects of the subject had to be left out entirely. It is sufficient to mention, for example, the chemistry of the antibiotics, other than penicillin, the mode of action of antibiotics upon bacteria, the increased resistance of bacterial strains to a given antibiotic, and others.

Various sciences have contributed to the development of the science of antibiotics, but none more than microbiology and chemistry. The resulting practical applications have been outstanding, but none has been more important than in the treatment of human diseases.

The contribution of the microbiologist to the science of antibiotics has proceeded along four distinct paths, which can be briefly summarized as follows:

1. **The Fate of Disease-producing Bacteria that Find Their Way Into the Soil.** With the rapid progress of medical bacteriology during the seventh and eighth decades of the last century, fear was expressed that pathogenic bacteria which find their way continuously into the soil may render the latter a source of infections and epidemics. A careful study, therefore, was undertaken of the bacterial population

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* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

of the soil and of the survival of pathogenic organisms in the soil. The results obtained from these investigations failed to substantiate the above assumption. On the contrary, it was found that the great majority of disease-producing bacteria rapidly die out in the soil. It was even suggested that this may be due to the activities of the native-soil microbiological population. Although a number of antagonistic organisms were isolated, and methods developed for measuring their antibacterial properties, no attempt was made, in most instances, to obtain the active agent in pure form and to apply it for the control of infections.

2. **Studies of Mixed Infections.** Beginning with the first observation of Pasteur, in 1877, that anthrax may not develop in infected animals when accompanied by common bacteria, it was repeatedly shown that mixed infections do not behave in a manner similar to infections caused by pure cultures of pathogenic bacteria. The non-disease-producing, or the saprophytic, organisms that accompany the pathogenic forms in the mixed culture modify considerably the action of the latter, and frequently repress them completely. Although Cantani first attempted, in 1885, to utilize this phenomenon for the control of infections, no important practical results were obtained from these observations, due to the impossibility of controlling the relative development of the pathogen and the saprophyte.

3. **The Gradually Accumulating Information Concerning the Microbiological Population of the Soil.** This information has yielded a better understanding of the specific composition of the complex microbial population, of the occurrence of various fungi, bacteria, and actinomycetes, and of the associative interrelations among the members of this population. These interrelations comprise both synergistic and antagonistic effects. It was shown that some of these effects can be utilized in the control of important plant diseases. The addition of certain forms of organic matter to the soil results in the development of a specific microflora which can keep in check the growth of certain plant pathogens.

4. **Accidental Observations of the Effects of One Organism upon Another, Growing upon a Culture Plate or in a Liquid Medium.** Numerous observations of this nature have been made by the plant pathologist, in his studies of the effects of one fungus upon another; by the soil microbiologist, in his observations of crowded plates made from soil suspensions; even by the medical bacteriologist, on bacterial plates contaminated, by chance or on purpose, by a fungus or by an actinomyces. Such observations frequently led to important deductions, as was the

case of the observation made by Fleming, in 1929, concerning the antibacterial action of a green mold, later identified as *Penicillium notatum.* This observation led Fleming to designate the active substance produced by the mold as *penicillin.* Although *penicillic acid* and *gliotoxin* have been isolated before penicillin, and although extracts of *Aspergillus fumigatus* have been utilized by Vaudremer, as far back as 1913, for the treatment of tuberculosis infections, neither of these observations and conclusions led to any such spectacular developments as those made by Fleming.

Thus, a considerable amount of information has gradually accumulated concerning the production by microorganisms of chemical substances, designated as *antibiotics,* which possess marked antibacterial properties. Some of these substances are formed by bacteria; others are produced by fungi; still others, by actinomycetes. Finally, when Dubos demonstrated, in 1939, that microorganisms can be isolated from the soil which have the capacity to produce, under certain conditions of culture, substances that not only possess antibacterial properties *in vitro,* but are also active *in vivo,* thus offering promise as agents to be utilized for chemotherapeutic purposes, a marked stimulus was given to the development of the subject of antibiotics. This led to a reexamination of the phenomena of antagonistic effects of various microorganisms, and to a comprehensive study of the production by microorganisms of antibacterial agents and their possible utilization for disease control. A number of antibiotic substances have been isolated. However, only two of these may be considered in further detail: namely, penicillin and streptomycin.

Although Fleming's original observation has commonly been referred to as accidental, it must be emphasized that he fully recognized that penicillin had a "strong inhibitory effect on many of the common bacteria which infect the human body, but which was not toxic to animals or to human leucocytes." He also visualized the potentialities of this substance, as shown by a further statement that "it is likely that it, or a chemical of a similar nature, will be used in the treatment of septic wounds." Fleming, however, did not isolate the new agent from the culture medium and failed to find immediate practical application for it. It remained in the medium, in a very dilute form, and no effort was made to determine its chemotherapeutic properties.

Florey, Chain, and their associates, comprising the Oxford group, must be credited with having established the potentialities of penicillin as an agent for the treatment of disease. This team of chemists, bacteriologists, and pathologists undertook a survey of the ability of various microorganisms to inhibit the growth of bacteria. In the words of the authors, "It occurred to two of them that it would be profitable to conduct a systematic investigation of the chemical and biological properties of the antibacterial substances produced by bacteria and molds." Out of the various antibiotics already known and recorded in the scientific literature, they gave first consideration to pyocyanase and penicillin. They soon discovered that the latter offered the greater promise. Suitable methods were developed for growing the fungus; for isolating the antibiotic agent, penicillin, from the medium; for testing its antibacterial properties; and for establishing that it can be used therapeutically, for the control of certain important human diseases. These investigators were also responsible for bringing to the attention of the American medical world and of American industrial organizations the great potentialities of this new antibiotic, as an agent for combating certain serious human diseases.

It remained, finally, for American industry to make possible the production of penicillin. The yield of penicillin in the medium, as a result of the growth of the fungus *Penicillium notatum,* was increased from 2–4 Oxford units per milliliter to 200 and 1,000 units per milliliter. New and very active strains of the penicillin-producing fungi were isolated. Finally, the deep or submerged method for the production of penicillin on a large practical scale was developed. Penicillin was isolated, crystallized, and its chemical nature determined. This remarkable development in the production of penicillin has, finally, led to a rapid rise in its use, on a scale never dreamed of even 3 or 4 years ago.

The development of streptomycin has taken place in somewhat different, although comparable, stages. The story of this antibiotic is that of a search for an agent capable of exerting bacteriostatic and bactericidal effects upon gram-negative bacteria, a substance active against these organisms, not only in the test tube but also in the animal body, yet not very toxic, nor exerting otherwise undesirable effects upon the body, a substance that would not be inactivated by the body fluids and that would, therefore, offer chemotherapeutic potentialities.

Soils, composts, and other natural substrates harbor large numbers of organisms capable of producing antibiotic substances. Some of these organisms form more than one type of antibiotic. These substances vary greatly, in chemical nature, in selective antibacterial properties, in toxicity to animals, and in their activity in the animal body. It soon became apparent that, in order to isolate the desirable kind of antibiotic substance, detailed surveys of large numbers of microorganisms would have to be made; suitable media for the growth of such organisms and proper methods for their isolation from the medium had to be developed. Within a period of five years, nearly ten thousand such cultures were examined, before streptomycin was obtained.

Although the question of the control of human and animal diseases caused by various bacteria may now be viewed with considerable optimism, it cannot be considered as having been solved. There still remain a number of problems that face the investigator and that require further elucidation. Chief among these, is the increasing resistance or fastness of certain strains of bacteria to given antibiotics, after prolonged contact. Thus, there may develop penicillin-resistant gonorrhoea strains, as well as staphylococcus and streptococcus strains. This phenomenon may prove to be of particular importance, in the case of chronic diseases like undulant fever or tuberculosis, where prolonged treatment with a single agent, such as streptomycin, may result in the development of resistant strains. Further, even the naturally occurring strains show marked variation in their sensitivity to a given antibiotic. Certain bacteria, like *Pseudomonas aeruginosa,* are themselves capable of producing antibiotic substances. This may be the reason for the greater natural resistance of this organism to the action of various antibiotics. A number of diseases, notably the many infections caused by viruses, are hardly affected by antibiotics.

The coordinated efforts of the microbiologist, the chemist, the pharmacologist, and the clinician, will finally lead to the control of these and many other diseases that are now plaguing mankind. The microbiologist will have made his contribution, by the isolation of new microbes, capable of forming non-toxic substances active against various disease-producing organisms; by developing methods for the optimum growth of these organisms and for measuring their antibacterial activities; by helping to elucidate the mode of action of antibiotics upon the disease-causing agents; and, finally, by evaluating the practical potentialities of antibiotic substances as chemotherapeutic agents.

The purpose of these papers is not only to summarize our present knowledge of antibiotics and their application, but to call attention to the problems that still await solution.

THE DEVELOPMENT OF IMPROVED PENICILLIN-PRODUCING MOLDS

By Kenneth B. Raper

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In reporting the discovery of penicillin, Fleming (1929) referred to the responsible mold as Biourge's *Penicillium rubrum.* Subsequently, Raistrick sent this culture to Thom,[35] who diagnosed it as closest to *Penicillium notatum* Westling. This corrected nomenclature was accepted by Raistrick and co-workers, and by Fleming, and was incorporated in their papers published in 1932. It likewise gave direction to the search for better penicillin-producing molds, when such a search assumed unexpected significance, following the work of Florey, Chain, Heatley, *et al.*, in 1940 and 1941.

All of the early studies on penicillin production were made with the original Fleming strain (plate 1, figure 2), and, during this period, the idea seems to have developed that this particular strain was unique in its capacity to produce penicillin. This view was questioned, soon after work was initiated at the Northern Regional Research Laboratory, in July 1941, since it was at variance with our experience in other mold fermentations (*e.g.*, citric and gluconic acids). Cultures belonging to the *Penicillium notatum-chrysogenum* group, then contained in the NRRL Collection (about 35 in number), were tested for their capacity to produce penicillin, and almost all proved to be positive (Moyer and Coghill[21]). No other strain was found to produce yields equal to the Fleming strain, when grown in surface culture, but a second strain of *Penicillium notatum*, NRRL 832, was found to produce greater yields than the Fleming strain, when grown submerged (Moyer and Coghill[22]). Yields with 832 in submerged culture were low, when compared upon a units/ml. basis with surface yields obtained from the Fleming strain, but the culture seemed to offer great promise, when considered from the standpoint of its industrial potential. Subsequently, it was generally adopted by industry and was employed, for many months, for the production of penicillin in deep culture. It was thus apparent: (1) that the capacity to produce penicillin as a metabolic product represented a group, rather than a strain or specific character; (2) that different members of the group varied greatly in their capacity to produce penicillin; and (3) that particular strains were especially suited for certain types of production.

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* This is one of four regional laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Concurrent with this development, it was found that good penicillin-producing strains were often subject to considerable variation in laboratory culture, and that substrains of differing productivity could be separated out and maintained in culture. Strain NRRL 1249.B21 (PLATE 1, FIGURE 3), of monospore origin, was developed from the Fleming culture, in this manner, in late 1942. This strain was noteworthy for its capacity to produce substantially increased yields of penicillin, when grown in surface culture (Moyer and Coghill[21]). It found immediate acceptance in this country and in Great Britain, and it is still employed, wherever penicillin is produced by this method. It has, also, been employed for the production of penicillin by the "bran-process," and for the preparation of surgical dressings, a development which attained some proportions in Hawaii and the South Pacific Area, during the war (Carpenter, Weller, and Martin[6]; Larsen[16]; and Agmar[2]). For detailed information regarding the sources and cultural characteristics of strains NRRL 832 and 1249.B21, the reader is referred to the paper by Raper and Alexander[28], on mycological aspects of penicillin production.

The search for better penicillin-producing molds was rapidly expanded in 1943. At the Northern Regional Research Laboratory, a program was undertaken to isolate from nature as many representatives of the *Penicillium notatum-chrysogenum* group as possible, and to test these for their capacity to produce penicillin (Raper, Alexander, and Coghill[27]). Strains were isolated from food products, fruits and vegetables, and from soils collected from numerous stations in the United States and in many foreign countries (the Air Transport Command assisted us materially in securing the latter). In the handling of these cultures, a simple screening test was developed, which effectively weeded out the less productive strains, while the more promising ones were studied thoroughly in surface, and in shaken-flask, cultures. A general correlation was observed between cultural and morphological characteristics and penicillin-producing capacity of these isolated cultures.[27] No strains were isolated from nature which exceeded NRRL 1249.B21 for the production of penicillin in surface culture, but a limited number of strains were found which produced yields in excess of NRRL 832, when grown submerged. Of these latter cultures, *Penicillium chrysogenum* NRRL 1951 (isolated from a cantaloupe collected in Peoria, Illinois) was found to be extremely variable in culture, and from it, a series of superior producing substrains was obtained, including NRRL 1951.B25 and the justly famous strains, X-1612 (see page 45) and Wis. Q-176. The capacity to produce penicillin, in surface and in submerged culture, was tested in lactose-steep liquor media, as subsequently reported by Moyer and Coghill.[21, 22] For detailed information, regarding the survey, the reader is referred to our original paper.[27]

An intensive study of natural variation in selected strains was also undertaken as a possible means of securing more productive types (Raper & Alexander[28]). Substrains of NRRL 1249.B21 were repeatedly isolated and tested, but none of these was found to be superior to the parent culture. Variation was principally in two directions: (1) increased sporulation and pigment production, suggestive of the original Fleming type; and (2) reduced exudate formation and decreased sporulation in the direction of sterile colonies. Yields of penicillin declined markedly, as the culture differed increasingly, in either direction, from the typical 1249.B21 type.* Attempts to isolate various substrains from NRRL 832 failed to produce any markedly superior penicillin-producing cultures, but did reveal some striking cultural variants, including one substrain characterized by a striking red to light purple coloration. Outstanding success was realized in the development of high-yielding strains from NRRL 1951 (PLATE 2, FIGURE 4). When first tested, this strain produced yields, in submerged cultures, slightly in excess of NRRL 832. Its potentialities became evident, when colonies in streak-plate cultures seeded from week-old shaken flasks developed conspicuous sectors, and when the subcultures from these were found to produce greatly increased yields, both in surface and in submerged cultures. Repeated cultivation and reisolation of new substrains, from dilution plates and from sector variants in giant colonies, yielded numerous high-producing strains. Of these, NRRL 1951.B25 (PLATE 2, FIGURE 5) appeared to possess a slight advantage, although it was neither culturally very different from some other isolates, nor was it a markedly better penicillin producer. This strain produced yields in surface cultures, equal to, or slightly in excess of, NRRL 1249.B21 and was less sensitive to temperatures above 24°–25° C., but its greater pigment production precluded its adoption by the surface culture industry. Its principal value stemmed from its behavior in submerged culture, where yields approximately double those for NRRL 832 were usually obtained. As in the case of NRRL 1249.B21, continued investigation of natural variations in strain 1951.B25 failed to reveal any subculture capable of producing higher penicillin yields than the parent stock, although the widest variety of cultural types was separated out and tested (Raper and Alexander[28]).

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* **Schatz, A., & S. A. Waksman**[32] have reported a similar reduction in streptomycin-producing capacity in non-sporulating strains of *Actinomyces griseus.*

Through a similar selection of natural variants, higher yielding substrains have since been developed from other basic stocks, such as *Penicillium chrysogenum,* Minn. R-13 (= NRRL 1984). In these cases, also, certain increased levels of productivity were reached, beyond which no further improvement was made, although the selection and examination of naturally occurring cultural variants were continued.

By the beginning of 1944, it was obvious that additional cultures, capable of producing satisfactory yields of penicillin, could, in all probability, be isolated from nature. It was likewise clear that processes of natural variation and selective subculturing could probably be utilized to secure more productive substrains from some of the new cultures thus obtained. The possibility of developing improved penicillin-producing cultures, through the production of induced mutations by means of ultraviolet and X-ray radiation, or other artificial means, appeared promising.* Possessing this information, and faced with the demand for ever-increasing amounts of penicillin, the Office of Production Research and Development of the War Production Board set up projects at the University of Minnesota, the University of Wisconsin, Stanford University, and the Carnegie Institution of Washington (Cold Spring Harbor, Long Island), to explore vigorously each of the above approaches. In this work, attention was directed, primarily, toward the development of better "submerged" cultures, since it was then obvious that this type of production was most feasible.

At the University of Minnesota, attention was directed, primarily, toward the isolation of new strains from soil and other natural sources. One culture of outstanding merit, Minn. R-13, was discovered, which has been widely studied and from which industrially important substrains have been developed, through natural variation and induced mutation (see, also, page 47). At the University of Wisconsin, strain variation was investigated by one group, and the metabolism of penicillin-producing molds by another. The far-reaching results of this work are being reported to this conference by Professor Johnson.[25] At Stanford University and at the Carnegie Institution, attention was directed, primarily, toward the production of artificially induced mutations from known good strains, such as NRRL 1951.B25, by exposing conidia to ultraviolet and X-ray radiations. At Stanford, alone, more than 60,000 isolates were tested, and a limited number of strains was found to produce somewhat better yields than the parent stocks. At the Carnegie Institution, a much smaller number of cultures was irradiated, but of these, one strain was produced which, for a time at least, warranted the title, "super strain," namely X-1612 (PLATE 3, FIGURE 6). The production of this culture should be regarded as a joint endeavor. The stock was supplied by the Fermentation Division, Northern Regional Research Laboratory; the irradiation was performed by Dr. Demerec and associates at the Carnegie Institution, in Cold Spring Harbor; the initial and indicative production tests were made at the University of Minnesota, by Drs. Christensen and Ehrlich; and the real magnitude of its superiority was demonstrated, at the University of Wisconsin, by Professors Peterson and Johnson in 80-gallon fermenters. To the microbiologist and chemist alike, it is of particular interest that strain X-1612 differs from the parent, primarily, in biochemical activity, and little, if at all, in cultural or structural detail. Maximum yields obtained with the parent 1951.B25 have ranged around 250 u./ml., while yields in excess of 500 u./ml. have been obtained with the mutant, X-1612 (Johnson[25]). Culturally and microscopically, the strain resembles closely the parent stock and, in the studies that we have made, it has been found to be equally variable in culture. Fortunately, in this strain, as in the parent, NRRL 1951.B25 (Raper and Alexander[28]), maximum production seems to be associated with a particular cultural aspect. Once this is recognized, the maintenance of productive stocks does not prove difficult.

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* In our own laboratory, improved itaconic acid-producing strains of *Aspergillus terreus* had been obtained from ultraviolet irradiated conidia.[14, 30, 18, 15] At Stanford University, **Beadle, G. W., & E. L. Tatum,**[4, 33, 34] and associates had produced a whole series of mutations in *Neurospora,* characterized by altered physiological and biochemical characteristics. It seemed entirely reasonable to hope that penicillin production might be enhanced by the blocking or altering of enzyme systems.

More recently, a much higher yielding strain has been developed from X-1612 by Professors Backus and Stauffer, at the University of Wisconsin. By irradiating conidia of X-1612 with ultraviolet, a mutant, designated Q-176 (PLATE 3, FIGURE 7), has been obtained, which is capable of producing yields of penicillin in excess of 900 u./ml., under favorable conditions (Backus, Stauffer, and Johnson[3]). Regarding the production of this outstanding mutation, Dr. Backus wrote (personal communication) as follows: "A monoconidial line was selected from X-1612 to be the 'parental' form for this irradiation series. A stirred standardized spore suspension of this race was irradiated at 2650 Å. The majority of the conidia perished. The survivors were allowed to germinate on plates and several hundred isolates were thus obtained.

When tested in shake-flasks, Q-176 gave us a yield very considerably higher than its parent............Q-176 was also tried out in the Biochemistry tanks and here also gave very much better yields than X-1612. In fact, the differential was greater than in shake-flasks."

Culturally and morphologically, strain Q-176 bears a striking resemblance to the parent strain, X-1612, and the "grandparent," 1951. B25. In agar plate cultures, there is an evident, but not particularly marked, progressive reduction in rate of growth from 1951.B25 to X-1612 to Q-176 (FIGURES 5, 6, and 7). But colony patterns remain essentially the same, and the production of atypical varieties of *Penicillium* as reported for 1951.B25 by Raper and Alexander,[28] is fairly consistent in both X-1612 and Q-176. Like the parent strains, 1951. B25 and X-1612, Q-176 is culturally unstable and tends to produce variants, differing in cultural aspects and in penicillin production. The strain represents, primarily, a biochemical, rather than a cultural or morphological, mutation.

We now know that members of the *Penicillium notatum-chrysogenum* group, even in the absence of special growth supplements, produce at least four different penicillins, known in the United States as F, G, X, and K (Coghill and Koch[8]), and that two or more of these may be produced in the same culture broth. Since these penicillins differ from each other in chemical characteristics and in their inhibitory effect upon susceptible bacteria, it has long seemed reasonable that they might possess different possibilities in clinical practice. Some supporting evidence has been reported. More than a year ago, Welch and associates[38] reported penicillin X to be more effective than commercial penicillin, which was primarily penicillin G, for the treatment of gonorrhea. More recently, Ory and associates[23] have reported various types of cocci to be from two to eight times more sensitive to preparations containing 65 per cent or more of penicillin X, than to commercial penicillin. Similarly, Libby and Holmberg[17] have found penicillin X more effective than penicillin G against a number of different bacteria, including various types of streptococci and pneumococci. Flippin *et al.*[11] reported the successful use of penicillin X, in the treatment of a case of bacterial endocarditis (*Streptococcus viridans*) which had failed to respond to repeated administrations of commercial penicillin.

Because of the anticipated possible therapeutic significance of this penicillin, and because of its interesting chemical possibilities, we set about, in March 1945, to secure, if possible, a culture capable of producing substantial yields of penicillin X in submerged culture. While penicillin X was first isolated from surface fermentations with NRRL

1249.B21, and while the material available for clinical use and chemical investigations, up to the present time, has come from this source, it was realized that a good "submerged" strain would be required, if penicillin X were to be made in quantity. Several of our best producing strains were investigated. Of these, NRRL 1984.A, a naturally-occurring variant of Minn. R-13, selected at the Northern Regional Research Laboratory, showed the highest ratio of penicillin X, amounting to more than 15 per cent by assay in drum fermentations, and up to 25 per cent in shaken-flask cultures. Attempts to develop natural variants, characterized by increased penicillin X production, were unsuccessful. By irradiating conidia of the same strain with ultraviolet (primarily, 2537 Å), however, a substrain, designated NRRL 1984.N22, was obtained that gave satisfactory total yields, of which approximately 50 per cent represented penicillin X (Raper and Fennell[29]). In 600-liter vat fermentations, total yields of approximately 200 u./ml. have been obtained in 90 hours. As indicated by differential assay, and as subsequently shown by actual isolation, penicillin X represented approximately 50 per cent of the total potency, as measured in *Staphylococcus* units/ml., or about 65–70 per cent of the total, upon a weight basis. Culturally and morphologically, this high penicillin X-producing mutant closely resembles the parent strain, and both fit reasonably well our species concept for *Penicillium chrysogenum* Thom.

Parallel with the work already reviewed, other investigators have made extensive surveys, searching for molds capable of producing penicillin or other valuable antibiotics. While tests have by no means been confined to the *Penicillia* and the *Aspergilli*, attention has been centered, quite naturally, upon these two abundant and cosmopolitan genera. Almost without exception, whenever penicillin, or penicillin-like substances, have been reported from molds, the species responsible have been found to represent one of these two genera. In view of the rapid developments in this field, no list can long remain up to date. Nevertheless, the following summary is believed to be currently complete, or nearly so: Penicillin or penicillin-like substances have been reported from *Aspergillus flavus,* by Bush and Goth[5] ("flavicin"); by McKee and MacPhillamy[19] and McKee, Rake, and Houck[20] ("flavicidin"); by Waksman and Bugie[37] ("flavicin"); and by Dr. R. G. Benedict, at the Northern Regional Research Laboratory (unpublished). Cook and Lacey[9] ("parasiticin"), and Benedict have likewise reported a penicillin-like substance from *Aspergillus parasiticus,* a species closely related to *Aspergillus flavus.* *Aspergillus oryzae* is listed as weakly positive, by Waksman and Bugie,[37] and as positive, by Foster and Karow.[13]

Philpott[26] reported a penicillin-like substance, "gigantic acid," from *Aspergillus giganteus*. Penicillin-like substances have also been reported from *Aspergillus flavipes*, by White,[39] Foster and Karow,[13] and Benedict; from *Aspergillus nidulans*, by Foster and Karow;[13] and from *Aspergillus nidulans* and the closely related species, *Aspergillus caespitosus*, by Benedict; from *Aspergillus niger*, by Foster and Karow;[13] and from *Aspergillus sydowi*, by Benedict and by Robbins (both unpublished).

Members of the *Penicillium notatum-chrysogenum* group characteristically produce penicillin in greater or less amount (Moyer and Coghill,[21] and Raper, Alexander, and Coghill[27]). For this reason, a listing of reported penicillin production by species belonging to this group as it is considered by Thom under the group name, *"Radiata"* (The Penicillia, pages 259–269[36]), is regarded as superfluous. A few additional species deserve more attention. Florey *et al.*[12] reported penicillin-like substances from *Penicillium avellaneum*, *P. rubens*, and *P. turbatum*, in addition to two members of the *P. notatum-chrysogenum* group, *P. baculatum* and *P. fluorescens*. At about the same time, Dr. L. J. Wickerham, of the Northern Regional Research Laboratory, using a bacterial spectrum plate technique, obtained positive results with *P. avellaneum*, *P. rubens*, and cultures believed to represent *P. lanosum*, *P. roseo-citreum*, and *P. griseo-fulvum*, while individual strains of *P. spinulosum* and *P. turbatum* were found to produce both penicillin and spinulosin (unpublished data). *Penicillium avellaneum* is a well-defined species and is not regarded as closely related to the *P. notatum-chrysogenum* group. *Penicillium turbatum* and *P. spinulosum* are monoverticillate forms and, hence, clearly different from *P. notatum*, etc. *Penicillium rubens* may warrant species recognition, but bears a striking resemblance to the *P. notatum-chrysogenum* group. *P. lanosum*, *P. roseo-citreum*, and *P. griseo-fulvum* have been regarded as distinct species and have been placed in different sections of the genus from *P. notatum*, etc. (Thom[36]), but the strains tested possess certain cultural characteristics (*e.g.*, colony pattern, pigmentations, and exudate formation) strongly suggestive of this group and may, in fact, belong with it. It is believed to be of real significance that, in the genus *Penicillium*, with the exception of *P. avellaneum* and the provisional exception of *P. spinulosum* and *P. turbatum*, penicillin and penicillin-like compounds have been reported only from members of the *P. notatum-chrysogenum* group and certain other additional species that are believed to be closely allied to it. In the genus *Aspergillus*, the production of penicillin-like substance seems to be more general, but appears to be most commonplace among strains belonging to the *Aspergillus flavus* group.

Outside of these genera, Peck and Hewitt[24] have reported the production of a penicillin-like antibiotic by the dermatophyte, *Trichophyton mentagrophytes.*

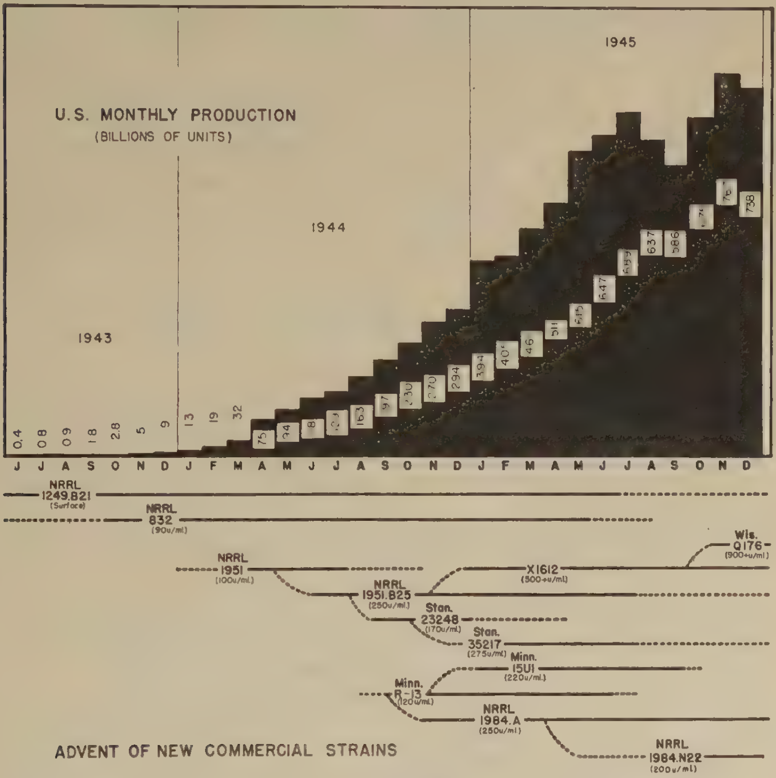

FIGURE 1. Diagram showing the general correlation between the monthly production of penicillin, from July 1943 to December 1945, and the development of new industrially important strains. In such a presentation, it is impossible to differentiate between increases in production, due to new facilities and improved operational practices, on the one hand, or to the advent of higher-yielding cultures, on the other hand. However, the contribution of the latter has been very substantial. Essentially the same culture solution is employed to obtain 400–500 u./ml., with X-1612, as was earlier employed to obtain 75–90 u./ml., with NRRL 832.

Extensive surveys have been made by Wilkins and Harris,[40–43] in England, and by Robbins *et al.*,[31] in this country, covering a wide variety of filamentous and fleshy fungi. Representatives of many species have been found to produce antibiotic substances, but, as yet, the activity and properties of these substances have not been adequately defined. As a result of additional studies now in progress, it may develop that some of these antibiotics represent penicillin and that we shall have to regard the formation of this metabolic product as a commonplace phenomenon. But, today, this prospect is not in sight. With one known exception, penicillin production appears to be limited to the *Aspergilli* and *Penicillia,* and is especially characteristic of a particular group within the latter genus. Inside this group, the number of basic strains that are outstanding is limited, and from these have been developed our present high-yielding substrains, by the selection of natural variants and by the production of artificially induced mutations. One cannot, of course, predict the source of high-yielding strains yet to be discovered. However, upon the basis of our present knowledge, it seems probable that they, too, will come from the *Penicillium notatum-chrysogenum* group, by an extension of techniques already in common use. (Note FIGURE 1.)

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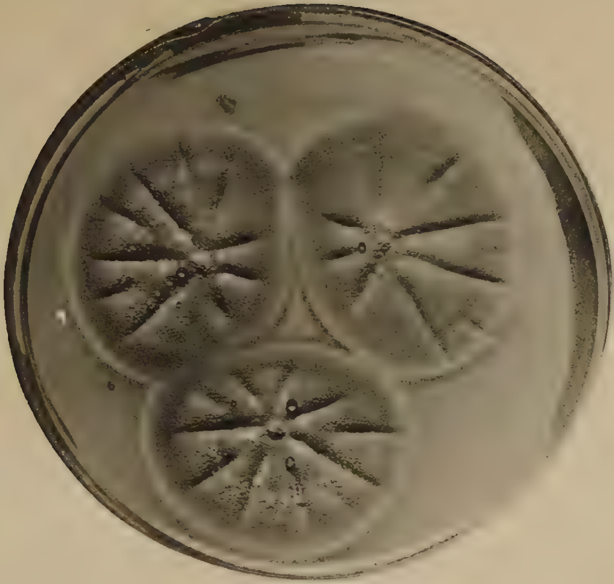
PLATES 1–3

PLATE 1

FIGURE 2. *Penicillium notatum,* NRRL 824, the unimproved Fleming strain, as used in early penicillin investigations.

FIGURE 3. *Penicillium notatum,* NRRL 1249.B21, an improved substrain developed from the Fleming culture. It was widely used for the production of penicillin, by the surface culture method.



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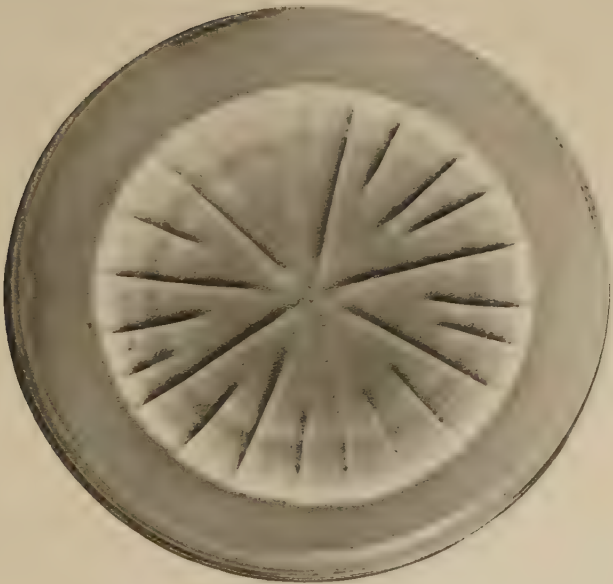

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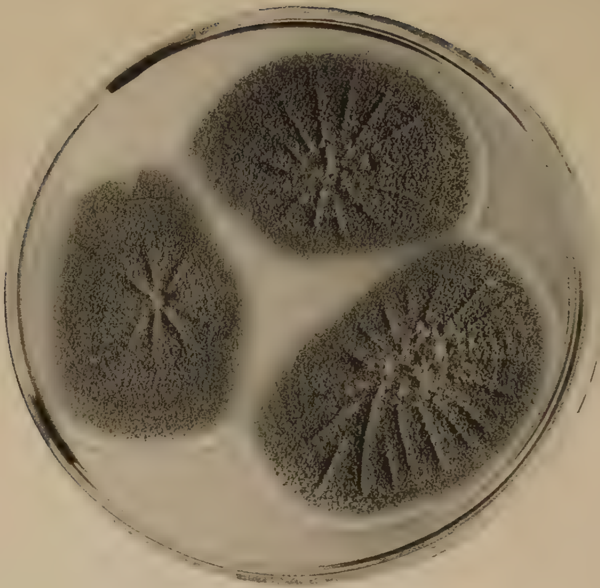
RAPER: DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS



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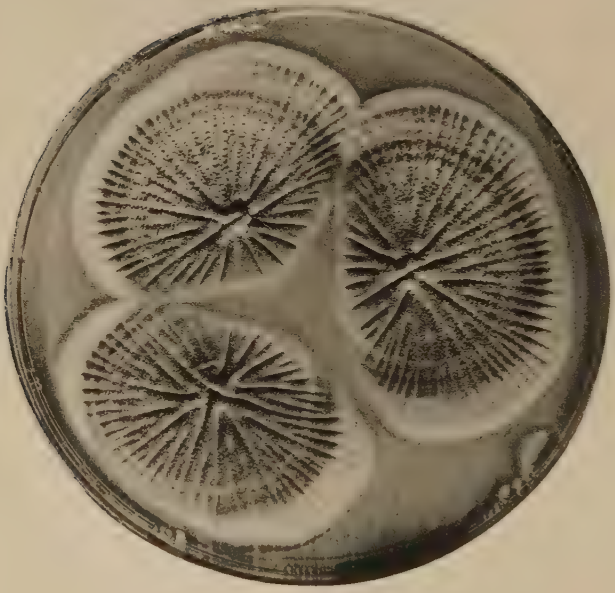

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RAPER: DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS

PLATE 2

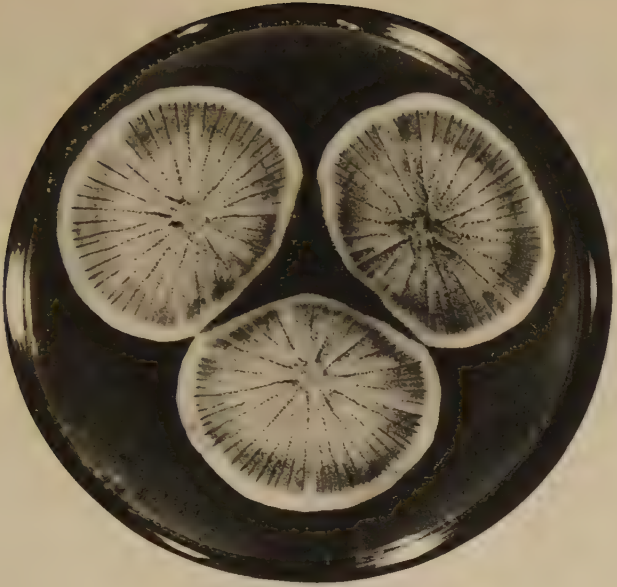
FIGURE 4. *Penicillium chrysogenum,* NRRL 1951, a strain isolated from a cantaloupe, at the Northern Regional Laboratory. Capable of producing approximately 100 u./ml. of penicillin, in submerged culture, it is of primary interest and importance as the source of greatly improved substrains.

FIGURE 5. *Penicillium chrysogenum,* NRRL 1951.B25, a natural variant of NRRL 1951, capable of producing up to 250 u./ml. of penicillin, in submerged culture.

PLATE 3

FIGURE 6. *Penicillium chrysogenum,* X-1612, an X-ray-induced mutation of NRRL 1951.B25, capable of producing more than 500 u./ml. of penicillin, in submerged culture.

FIGURE 7. *Penicillium chrysogenum,* Wis. Q-175, an ultraviolet-induced mutation from X-1612, capable of producing more than 900 u./ml., of penicillin, in submerged culture.



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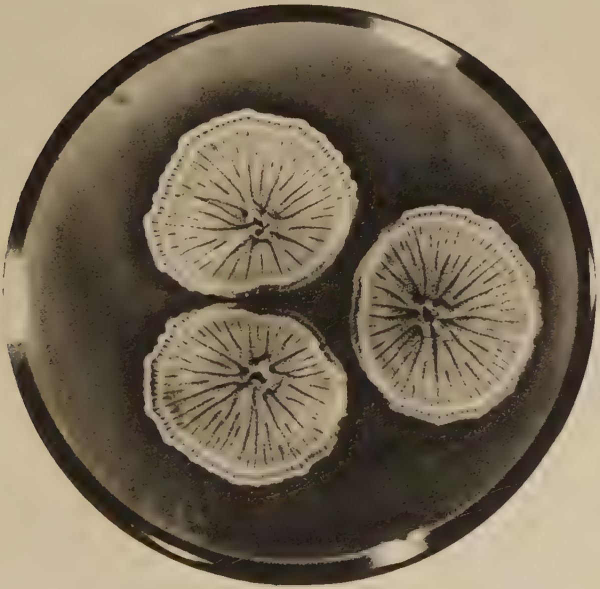

7

RAPER: DEVELOPMENT OF PENICILLIN-PRODUCING MOLDS

METABOLISM OF PENICILLIN-PRODUCING MOLDS

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Standard procedure,[1, 2, 3] in penicillin production, involves growing a mold of the genus *Penicillium* on a medium containing a carbohydrate source (usually lactose) and a source of nitrogenous material (usually corn steep liquor). "Synthetic" media, in which inorganic nitrogen sources are employed, have been used, but have not been found to give yields, in large scale fermentations, equal to those obtained on steep liquor media. The mold grows throughout the medium, and must be kept supplied with an adequate amount of air. In laboratory flasks, aeration may be accomplished by continuous shaking, but, in larger containers, stirring and aeration are used.

There is a definite and intimate relationship between the amount of penicillin yielded by the fermentation and the chemical changes, in the growth medium, brought about by the metabolism of the mold. The penicillin yield, with a given culture, is a function of such variables as pH, available oxygen, rate of carbohydrate oxidation, and available combined nitrogen. The reason for the superiority of one medium over another can usually be correlated with the differences in metabolic changes occurring in the two media.[3] The superiority of one level of aeration over another is also closely correlated with the changes in metabolism caused by variation in air supply. A study of the chemical changes occurring in the medium, during the penicillin fermentation, is, therefore, of interest, not only from a scientific standpoint, but also from the viewpoint of the industrial producer of penicillin.

AERATION

One of the factors which influence the quantity of air needed in submerged *Penicillium* fermentations is the rate of oxidation of carbohydrate. Table 1 gives data on the oxidation rate attained in shaken flasks, when glucose, sucrose, and lactose are used as carbohydrate sources. It will be noted that glucose is most rapidly fermented by the mold, and that the oxygen demand of the culture is also greatest

TABLE 1

RATE OF SUGAR FERMENTATION AND OXYGEN UTILIZATION BY *Penicillium notatum* CULTURES

Carbohydrate	Maximum rate of sugar fermentation*	Maximum 0_2 uptake**
	gm./l. per hr.	ml. 0_2 per hr./l.
2% Lactose	0.32	109
4% Lactose	0.36	134
4% Sucrose	0.46	150
4% Glucose	0.71	300

* Measured in shaken flasks in a steep liquor medium (culture 832).
** Measured on a sample of culture in a Warburg respirometer.

when glucose is used. Sucrose is used more slowly than glucose; lactose is most slowly utilized and gives the lowest oxygen demand. It might be expected that successful fermentations, with glucose as the carbon source, would require more aeration than is necessary for lactose fermentations. That such is the case, is shown by the data of TABLE 2.

TABLE 2

PENICILLIN YIELDS ON FLASKS IN ROTARY AND RECIPROCATING SHAKERS
(*Penicillium notatum* No. 832)

Medium	Vol. per flask	Reciprocating shaker (90 R.P.M.)		Rotary shaker (285 R.P.M.)		Rotary shaker (325 R.P.M.)	
		Pen. yield	Day of max. yield	Pen. yield	Day of max. yield	Pen. yield	Day of max. yield
	ml.	u./ml.		u./ml.		u./ml.	
4% lactose, 2% steep liquor solids	50	75	8	62	8	16	2
	100	75	7	71	8	...	...
	150	65	7	70	8	...	...
4% glucose, 2% steep liquor solids	50	21	9	35	4	61	3
	100	18	7	31	7	...	...
	150	10	8	26	8	...	...

In the experiment summarized in the table, two shakers were used: a conventional reciprocating shaker, in which the flasks were moved, horizontally, a stroke distance of 10 cm., at 90 cycles per minute, and a shaker in which the flasks described a horizontal circle, one inch in diameter, at the frequencies given in the table. The rotary shaker was designed to give a higher aeration rate than was possible with the reciprocating shaker. It will be noted that, when the lactose medium was used on the reciprocating shaker, the penicillin yield was essentially independent of the volume of medium in the flask, but, with glucose, yields were lower, and decreased when the air available per milliliter of medium was decreased by increasing the volume in the flask. When the rotary shaker was used at 285 R.P.M., yields on lactose were not affected, while yields on glucose increased. When the speed of the shaker was further increased, the yields on glucose became still better, and yields on lactose decreased. A comparison of the chemical changes taking place in glucose and lactose media will be considered later. For the present, it is sufficient to point out that penicillin yield is a function of the carbohydrate source used and of the amount of oxygen available to the fermentation.

In tank fermentations,[2] the air available to the culture is, of course, only a small fraction of the total air supplied to the tank. It is very difficult to produce and maintain air bubbles in a fine state of division, in a tank filled with a heavy growth of mycelium. The consistency of the mycelial mass is such that coalescence of bubbles is promoted. This is especially true of large tanks, while, in small tanks, the limited length of bubble rise decreases the effectiveness of oxygen absorption. In commercial tanks, the percentage used of the air generally varies from a few per cent to about 15 per cent. In the small tanks, in our laboratory, one per cent utilization is usual. A method which may be used to obtain an estimate of the aeration level required by a fermentation, is a determination of the amount of air used, at a number of levels of air supply. Since carbon dioxide produced is more conveniently determined than oxygen used, the rate of carbon dioxide production by the fermentation is determined as a function of the amount of air supplied to it. In FIGURE 1, data obtained in such a manner are plotted. The tank used contained 200 liters of medium. It will be seen that, at aeration rates greater than one volume per volume of culture per minute (200 liters per minute), very little increase in metabolism rate occurred, but lowering the air supply decreased the metabolism rate. It might be concluded that an aeration rate somewhere in the neighborhood of 200 liters per minute would be adequate. When an experiment, similar to that of FIGURE 1, is conducted on a large industrial penicillin fermentation, it is often found that, at high aeration rates, the efficiency of aeration decreases, and the energy input to the agitator decreases. This phenomenon, apparently due to flooding of the agitator with air, has been known to be so pronounced that an increase in aeration rate actually caused a decrease in metabolism rate. From FIGURE 1, it may be seen that a metabolism rate of 10 volumes of carbon dioxide per 1000 volumes of culture per minute may be reached with adequate aeration. In many commercial installations, the metabolism rates encountered are much lower, presumably because of the difficulty of maintaining adequate air dispersion in large tanks.

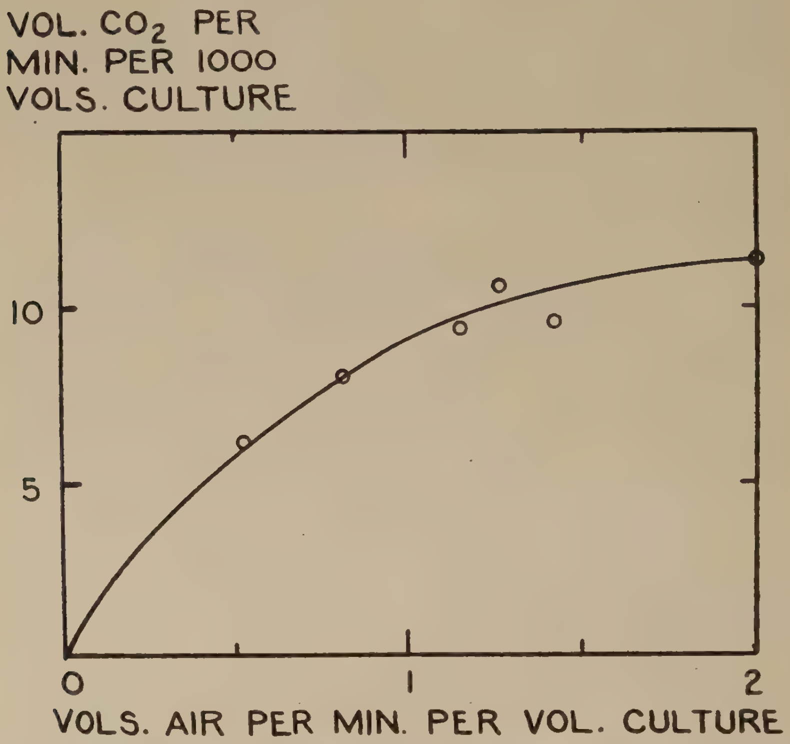

FIGURE 1. Effect of air level on metabolism rate in a tank fermentation. Culture 1951. B25 was grown on a medium containing 4% steep liquor solids, 3% lactose, and 1% calcium carbonate. Measurements were made when the culture was 20 to 23 hours old. The culture volume was 200 liters.

The data of FIGURE 1 were obtained by varying the aeration rate of a single fermentation. When complete fermentations are conducted at various aeration rates, similar results are obtained. The metabolism rate, during the fermentation, depends on the aeration rate. The penicillin yields, at low aeration rates, are definitely lower than those at high rates. In TABLE 3, a comparison is made of the metabolism and penicillin yield, obtained with different aeration levels. It will be noted that the lowest aeration rate used resulted in a delay in the growth phase of the fermentation, as evidenced by the later occurrence of the ammonia maximum.

It should be emphasized that the aeration level necessary in our tanks has only a very general relation to the aeration requirement of tanks of a different size and design. Factors such as sparger design, agitator design, and depth of tank influence the effectiveness of aeration so greatly that, perhaps, the only valid comparison that can be made is a comparison of metabolism rate. Even such a comparison is of limited usefulness, because many factors, other than aeration rate, may influence the rate at which oxygen is used.

TABLE 3

EFFECT OF AERATION RATE ON TANK FERMENTATIONS

Culture X-1612 was grown on a medium containing 3% lactose, 4% steep liquor solids, and 1% calcium carbonate.

Aeration	Penicillin yield (70 hours)	CO_2*	Age at max. NH_3 level
vol./min.	units/ml.		hrs.
0.15	278	2.7	47
1.0	490	8.9	25
1.5	497	10.0	26

* Volume CO_2 per min. per 1000 vols. medium, average 20th to 60th hour.

pH CHANGES

It is generally accepted that pH values much below 7 or much above 8 are not conducive to rapid penicillin formation. To what extent low or high pH values are secondary effects of conditions unfavorable to penicillin formation, and to what extent the acidity or alkalinity *per se* is effective, are not known. Most of the factors causing pH changes in penicillin fermentations in corn steep liquor media are known, and the pH can, to a large extent, be controlled.

When serial analyses are made on a penicillin fermentation, most of the factors influencing pH become obvious.

In FIGURE 2, analytical data on a fermentation in shaken flasks are plotted. Growth of the mold, expressed in the figure as mycelial nitrogen, takes place, first, at the expense of nitrogen-containing carbon compounds of the corn steep liquor. The soluble organic nitrogen decreases sharply, for the first two days. During this period, very little lactose is used, but good mycelial growth is obtained. Most of the nitrogen of the compounds used is turned into mycelial nitrogen, but a part of it accumulates as ammonia. After the second day, when the fermentable steep liquor nutrients are substantially exhausted, utilization of the less easily fermentable lactose begins. When lactose carbon is used to form cellular material, the nitrogen in this cellular material must arise from ammonia, since it is the only available nitrogen source. Hence, during lactose utilization, the ammonia concentration falls When the lactose is exhausted, no available nutrients remain, an

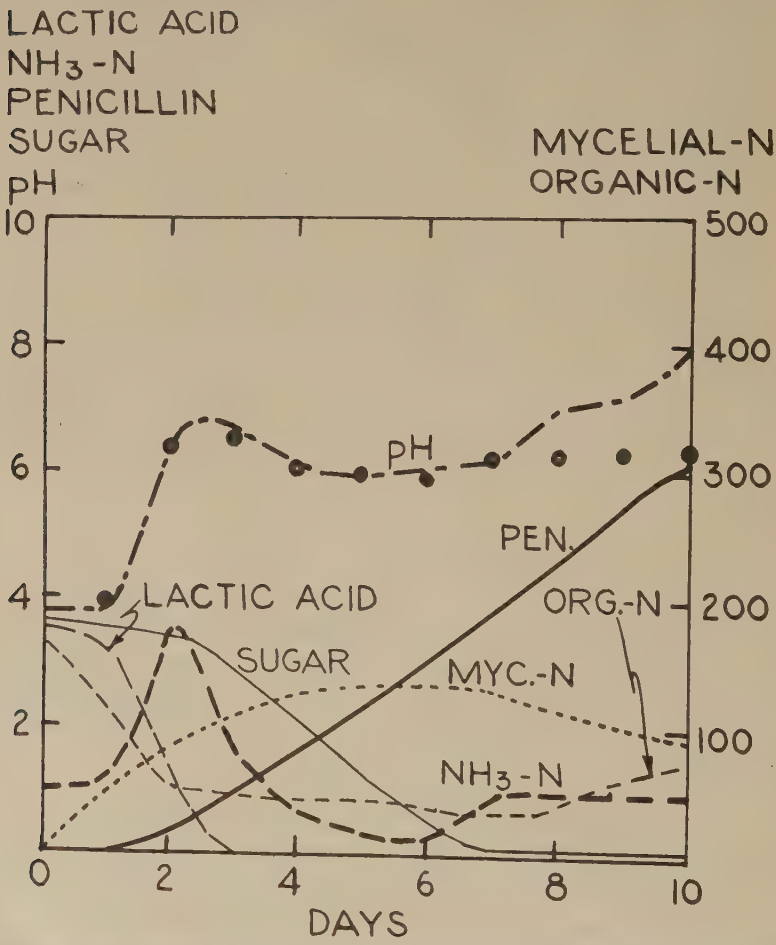

FIGURE 2. Chemical changes in shaken flask fermentations. Culture 832 was grown on a medium containing 3% lactose and 2% steep liquor solids. Penicillin is expressed as units per 0.1 ml.; mycelial N and organic N. as mg. per 100 ml.; lactic acid, as mg. per ml.; NH_3-N, as mg. per 10 ml.; and sugar, as g. per 100 ml.

autolysis begins. Mycelial nitrogen falls, ammonia rises slightly, and there is a sharp rise in soluble organic nitrogen compounds. Corn steep liquor contains considerable amounts of lactic acid. This lactic acid is intermediate, in availability, between steep liquor nitrogen compounds and lactose, since it begins rapidly disappearing when most of the available steep liquor nitrogen compounds have been used, and is completely fermented before very much of the lactose has been used. During the second day of the fermentation, ammonia is being accumulated and lactic acid is being used. Both processes result in a decrease in hydrogen ion concentration. The pH, therefore, rises sharply. After the lactic acid has disappeared, the utilization of ammonia, during the lactose-fermenting phase, results in a fall in pH. When the ammonia has been substantially exhausted, the pH does not change significantly until the onset of autolysis, when a sharp rise occurs.

The titration curve of the buffer mixture, in the steep liquor sample used in the experiment of FIGURE 2, was determined experimentally, and from it, the pH values which should have resulted from the observed changes in lactic acid and ammonia concentrations were calculated. These calculated pH values are plotted as circles, in the figure. They agree very well with the observed pH values, up to the time when autolysis began. This indicated that, before the onset of autolysis, the only factors causing pH changes are those here discussed, but that, during autolysis, another factor, probably liberation of organic bases from the mycelium, is operative.

METABOLIC CHANGES IN TANK FERMENTATIONS

FIGURE 3 gives analytical data on fermentations carried out in experimental tanks containing 55 gallons of medium. The curves represent the average analytical figures for three fermentations on a medium consisting of 4 per cent lactose, 4 per cent corn steep liquor solids, and 1 per cent calcium carbonate. The culture used was *Penicillium chrysogenum* X-1612, and the aeration rate was one volume of air per volume of medium per minute.

Comparison of the metabolic changes in tanks with those in shaken flasks shows that the fermentation is much more rapid. This is, to some extent, due to the larger inoculum used, but is chiefly the result of the more adequate air supply. It will be noted, although the nutrient level used was twice that used in the fermentation in FIGURE 2, utilization of the nutrients takes place in much less time. The first phase of the fermentation, during which mycelial growth takes place at the expense of the steep liquor nutrients, and during which the pH rises, because of lactic acid oxidation and ammonia liberation, lasts about 24 hours. The amount of mycelium formed, because of better aeration and because of the higher nutrient level, is greater, in the case of the tank fermentation. The use of high steep liquor concentrations is not advantageous in flask fermentations, because the air supply is inadequate to permit its utilization.

As in the case of the flask fermentation, penicillin is rapidly formed during the sugar-utilizing phase of the fermentation. The high penicillin yield in the tank fermentation is due, in part, to the use of a better culture and, in part, to the better air supply in the tank. It should be noted that, in FIGURE 3, penicillin concentration is plotted on a scale different from that used in FIGURE 2. FIGURE 3 does not give data extending to the autolysis period of the fermentation. This phase does not begin until available sugar is exhausted. In other tank fer-

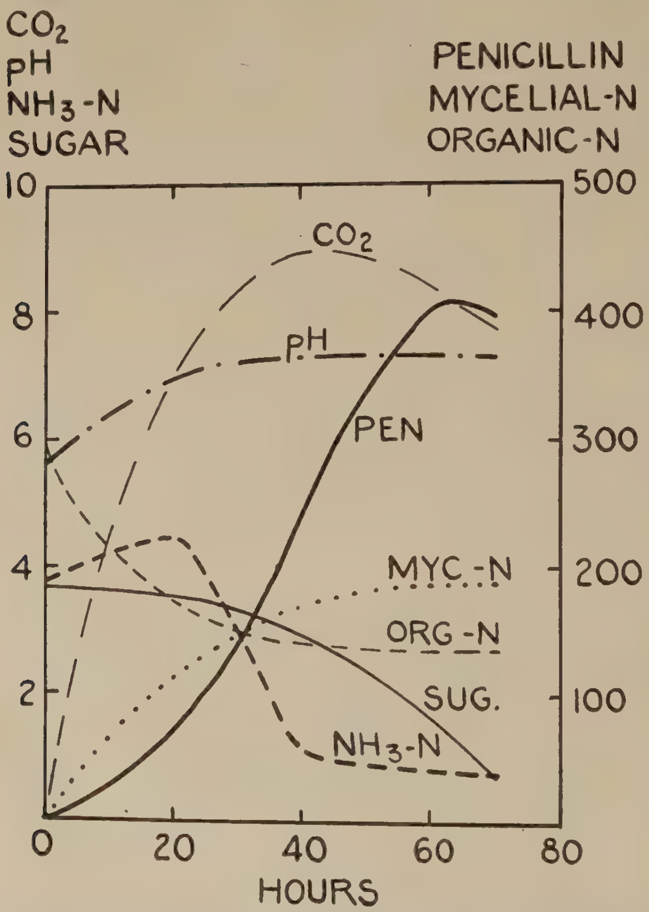

FIGURE 3. Chemical changes in tank fermentations. Culture X-1612 was grown in a medium containing 4% lactose, 4% steep liquor solids, and 1% calcium carbonate. Penicillin is expressed as units per ml.; mycelial N and organic N, as mg. per 100 ml.; NH_3-N, as mg. per 10 ml.; sugar, as gm. per 100 ml.; and CO_2, as volumes per min. per 1000 volumes culture.

mentations, when sugar exhaustion has occurred, autolysis, resulting in a rise in pH and an increase in ammonia level, has always taken place. We have never observed, in tank fermentations, an increase in penicillin concentration after the fermentable sugar has been depleted.

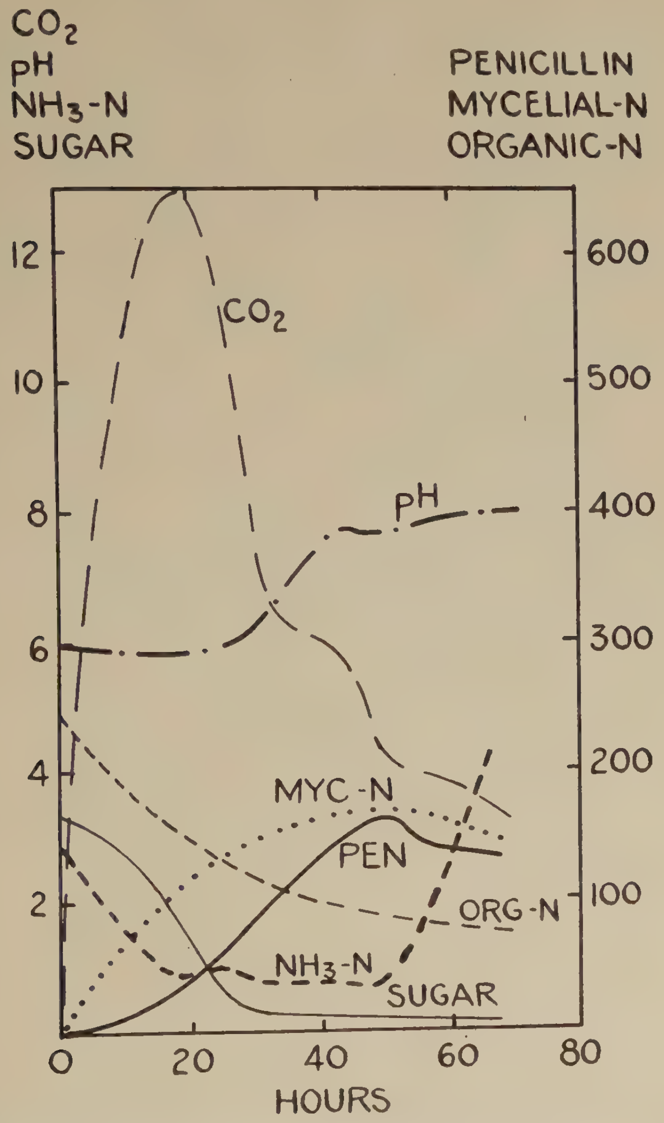

FIGURE 4. Chemical changes in a glucose medium. The experimental conditions were identical with those of FIGURE 3, except that glucose was substituted for lactose. The curves are drawn to the same scale as those of FIGURE 3.

USE OF GLUCOSE IN TANK FERMENTATION

It might be expected that, if lactose were replaced in tank fermentations by the more readily available glucose, a more rapid fermentation would result. It might, also, be expected that, because of its ready availability, glucose would be depleted sooner than lactose.

In FIGURE 4, the analytical data from a glucose tank fermentation are summarized. It may be seen that the metabolism rate, as measured by the rate of carbon dioxide production, is much higher, during the early stages of the fermentation, than the metabolism rate of the lactose fermentation. Glucose, as well as steep liquor, is utilized during the mycelium-forming phase of the fermentation. The nitrogen assimilation by the mycelium which accompanies the assimilation of glucose carbon, brings about a reduction, rather than an increase, in ammonia concentration, during the first phase of the fermentation. Since glucose is more available than lactic acid,[3] lactate utilization is delayed until after glucose utilization is completed. Because of the lack of ammonia production and because of delayed lactate utilization, there is no pH rise until the glucose is exhausted. There is, then, a sudden pH rise, after which, because of the lack of nutrients, autolysis begins. The pH plateau, during which, in the lactose fermentation, most of the penicillin is formed, is lacking in the glucose fermentation. The low penicillin yields with glucose media, in tanks, may be attributed to the lack of a period in the fermentation, during which pH and nutrient conditions are favorable for penicillin production.

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PRODUCTION OF ANTIBIOTIC SUBSTANCES BY BASIDIOMYCETES

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There are in the neighborhood of 100,000 species of known fungi. These are divided taxonomically into four groups, the Phycomycetes, Ascomycetes, Basidiomycetes, and the Fungi Imperfecti. Since the discovery of penicillin, a great deal of attention has been given to the production of antibiotic substances by the *Penicillia*, the *Actinomycetes, Aspergilli,* and other genera belonging, for the most part, to the Ascomycetes or to the Fungi Imperfecti. Relatively little has been done with the Basidiomycetes, comprising some 30,000 species and including the shelf fungi, puff balls, toadstools, mushrooms, and so on.

Folklore suggests that some of the Basidiomycetes may contain antibacterial substances. Agaric acid (N-hexadecyl-citric acid) obtained from *Polyporus officinalis* has been used, for many years, in the treatment of nightsweats of phthisis, and long chain aliphatic acids are antibacterial towards *Mycobacterium tuberculosis.* We have had reports that natives in Central America use the spores and skin of one of the earth-stars, to accelerate the healing of wounds. Rumor has it that woodsmen have long used the felt-like mats of basidiomycete mycelium, growing on trunks or logs, for bandages; whether this use is because of their cloth-like character, or because of hypothetical healing power, is uncertain. While it is unwise to discard reports of this character without consideration, they should be viewed with skepticism and require confirmation and extension by actual experiment.

Wilkins and Harris recently tested the antibacterial activity of extracts of the sporophores of 722 species, in 96 genera of the larger Basidiomycetes. About 1/5 of these were active against *Staphylococcus aureus* or against *Staphylococcus aureus* and *Escherichia coli.* None was active on *Escherichia coli* alone. About 30 species were grown in culture and discs of mycelium tested on seeded agar plates. Some of the 30 were active on *Staphylococcus aureus* or on *Staphylococcus aureus* and *Escherichia coli,* none on *Escherichia coli* alone. Wilkins and Harris did not determine the ability of their organisms to produce antibacterial substances in culture liquids.

Bose has reported that the filtrate of a species of *Polyporus,* in Czapeks-Dox medium, is active on *Escherichia coli* and the causal organisms of typhoid, cholera, and dysentery. Animal tests showed the filtrate which he calls polyporin to be "completely nontoxic."

Our own experiments, begun in June 1943, have included about 300 species in 42 genera. These were grown individually on agar in petri dishes and examined by the streak method for antibacterial activity, using *Staphylococcus aureus* and *Escherichia coli* as test organisms. About 114 of the fungi showed no activity, under our conditions. Of the balance, 29 produced zones of inhibition of less than 5 mm. on *Staphylococcus aureus;* 57, zones of between 5 and 10 mm.; and 119 zones of 10 mm. or more. Some affected *Staphylococcus aureus,* but not *Escherichia coli.* Some affected both. None was active on *Escherichia coli* alone. Forty-two of the more active Basidiomycetes, as tested by the streak method, were selected from those which were not strong acid producers and tested by the disc method. Sixteen of the forty-two were moderately or strongly active, as determined by the disc method. Some 25 of these fungi have been grown in culture liquids, and the antibacterial activity was determined by the dilution method against *Staphylococcus aureus.* Culture liquids, active at dilutions of between 250 and 1000, have been obtained from six. We are extending our studies to other Basidiomycetes, attempting to concentrate and isolate the active principles and examine their effects on other organisms.

I shall not attempt to describe our investigations in detail, but shall limit myself to some generalities which, we think, can be drawn from our experience thus far.

It seems evident that a considerable proportion of the Basidiomycetes produce antibacterial substances. Wilkins and Harris found 20% of 722 species to have such substances in their sporophores. About ⅔ of the 300 species we examined inhibited the growth of *Staphylococcus aureus,* in the vicinity of the fungus colony, and about ⅓ produced zones of 10 mm. or more. Of the 119 which exhibited marked activity on the streak plates, only 30 produced sufficient acid to account for their antibacterial action. In other words, of 300 Basidiomycetes, we found 89 which evidenced considerable antibiotic effects, not caused by the hydrion concentration of their products.

From our results with the streak method, the production of antibacterial substance did not appear to be associated with any particular taxonomic groups. One or more species, of 36 genera of the 42 included in our survey, showed some antibiotic activity, and the 6 which showed none were represented by only 1 or 2 species. Of 94 species and isolations of *Polyporus,* studied by the streak method, 51 were inactive; 19 gave inhibitions of 10 mm. or less; and 24, of 11 mm. or more. Of the 24, we considered the activity of 11 to be caused by acid production. Of 47 *Fomes* surveyed, 15 gave negative results; 13, inhibitions of 10 mm. or less; and 19, of 11 mm. or more. The antibiotic activity of 7 of the 19 was probably caused by acid formation. Of 41 *Porias,* 9 were inactive, while 19 produced inhibitions of 11 mm. or more. The antibiotic activity of 11 of the 19 appeared to be caused by acid.

While useful for survey purposes, the streak method has some distinct limitations.

It detects those substances, only, which diffuse from the mycelium. An antibiotic substance produced and retained in the body of the fungus will not be found. It is possible that some of the fungi we studied produce intracellular antibacterial substances. These would not be discovered by our survey method. The media used must be those which will support growth of the test organisms, as well as the fungus, unless a coating layer, adapted to the test organism, is used before applying the test organism. The character of the medium is frequently important, in determining the antibiotic activity of a fungus. For example, some of our fungi were quite active, when grown on a peptone-dextrose agar, but showed no activity on malt agar. Another factor of importance is the production of acid by the fungus. This may influence the activity of an antibiotic substance, even though the amount of acid formed is in sufficient *per se* to inhibit the test organisms. Furthermore, the streak method is not necessarily a guide to the effectiveness of a fungus. Some of the most active of our organisms, those which produced inhibition of *Staphylococcus aureus* at distances of 40 or 50 mm. from the fungus colony, showed little or no activity when tested by the disc method. It appeared that the quantity of the antibiotic material, which diffused into the medium from the fungus colony, was sufficient to inhibit the growth of the test bacteria, but was not large enough in an agar disc to give a zone of inhibition.

In judging the production of antibiotic substances, the test organisms are highly important. This is generally recognized, and it is as true for the Basidiomycetes as it is for other groups of fungi. Some of them inhibit *Staphylococcus aureus,* but not *Escherichia coli.* Some affect *Mycobacterium phlei,* but not *Escherichia coli* or *Staphylococcus aureus.* Others inhibit all three, and some inhibit other molds.

We have found the disc method useful for selecting fungi, among those found to be active by the streak test, for further investigation.

The disc method is more stringent than the streak method, because, in the latter, you deal with the product of an entire fungus colony; in the former, with the material in a small disc of agar or agar and mycelium —in our experiments, a disc 5.5 mm. in diameter. It has the further advantage of indicating where the greatest amount of antibiotic material is to be found, whether within the limits of the fungus colony, or outside. Furthermore, the discs from any medium on which the fungus may be grown can be tested on a neutral medium, of such composition as to be eminently suitable for the growth of the test organisms and unaffected by the growth of the fungus.

As might be anticipated, the results obtained with the disc method depend, in part, on the size of the disc; the larger the disc, the greater the quantity of antibiotic material. They depend also on the time between the application of the disc and the development of the test organisms. The character of the inhibition was found to vary with the fungus. In some instances, the disc was surrounded by a sharply-defined clear zone, indicating complete inhibition of the test bacteria. In others, the zone of inhibition was limited by a zone of stimulation. Discs from other fungi caused partial inhibition only, and the area of inhibition contained many small colonies or a few large colonies of a resistant strain. The medium on which the fungus grew affected the character of the inhibition zone.

The final test on any organism in which there is interest, from the standpoint of antibiotic substances, is to have it produce enough of the substance to permit concentration and, perhaps, isolation, for chemical investigation and for test on animals or other organisms. This cannot be done by growing the fungus on agar plates, nor, in most instances, by collecting sporophores in nature. It requires mass cultivation of the fungus in liquid culture.

For these purposes, the Basidiomycetes have some disadvantages. Most of them produce few or no spores, except basidiospores, and mycelium must be used for inoculation. Inoculation is less simple and less satisfactory than for the Penicillia, which produce spores freely and in quantity. Many of the Basidiomycetes also have marked growth-substance deficiencies and grow little, or not at all, on a simple synthetic medium, but require the presence of vitamins or extracts of natural origin. Of 310 Basidiomycetes, we found 190 to grow poorly, or not at all, on a synthetic medium, although all grew on malt agar. Of 91 species or isolations of *Polyporus,* 62 showed marked growth-substance deficiencies; of 32 *Porias,* 17; and of 45 *Fomes,* 20 were similarly affected. Some of them grow so slowly on any medium we have been able to discover, that the investigation of quantity production of antibiotic material is a slow process. Many of the slow-growing forms appear to be the most active. In fact, we have reached the point where we are almost prepared to discard any rapidly-growing Basidiomycete, without testing it.

This question of the relation between rapidity of growth, in these organisms, and production of antibiotic materials has intrigued us. While the relation is not strict, and there are a sufficient number of exceptions to eliminate it as a rule, it occurs sufficiently frequently to arouse one's curiosity. In fact, in many instances, we have observed the same organism to show much more activity on a medium on which it grows slowly, than on one on which it grows rapidly.

There seem to be two possible explanations for this phenomenon. Either the antibiotic substance inhibits the growth of the fungus itself, or an incomplete metabolism produces products which, in some instances, are antibacterial. We are inclined to believe that the latter is the correct explanation. For these reasons, the production of antibiotic substances in the culture liquid is not as simple a procedure as for *Penicillium notatum* or species of *Aspergillus.*

For these organisms, masses of asexual spores can be used as inoculum, and, if desired, the culture liquid can be limited to the mineral salts and sugar found in the modified Dox medium. Furthermore, they grow more rapidly in culture than most of the Basidiomycetes. Nevertheless, it has been found possible to produce active antibiotic culture liquids from some of the Basidiomycetes, and to concentrate the active principles to the point where a dilution, of from 1 to 40,000 up to 1-5,000,000, inhibits the growth of *Staphylococcus aureus* in broth cultures. We do not know whether any of these are of any therapeutic value.

The substances with which we are working are different from penicillin and differ from one another. It seems probable that a considerable number of new antibiotic substances is formed by this group. One means of determining this fact is the use of resistant strains of *Staphylococcus aureus.* In some instances, these resistant strains can be obtained from colonies in the vicinity of a fungus, in a streaked plate, or in the vicinity of a disc, on a seeded plate. In others, it is necessary to develop the resistant strains by cultivation of the stock strain in broth fortified by an amount of the antibiotic concerned which is partially, but not completely, inhibitory. Our experience with resistant strains of *Staphylococcus aureus* leads us to believe that they are useful for distinguishing between antibiotic substances, but do not serve to establish the identity of any two. This follows, because it is possible to develop strains resistant to two or more antibiotic substances.

It seems clear that many of the Basidiomycetes produce substances which, at considerable dilution, inhibit the growth of some kinds of bacteria. We have no evidence for the production of any substance as active as penicillin. On the other hand, we have investigated only about 300 of the 30,000 species in this group, and one of the 29,700 which remain may produce a substance with the activity of penicillin.

PRODUCTION OF ANTIBIOTIC SUBSTANCES BY ACTINOMYCETES*†

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It has now been definitely established that a considerable proportion of all actinomycetes that can be isolated from soils or other natural substrates have the capacity of inhibiting the growth of, and even of destroying, bacteria and other microorganisms.[5] This was brought out emphatically in several of the surveys that have been made on the distribution of antagonistic properties among actinomycetes.[1, 2, 4, 6, 10] Twenty to 50 per cent of all the cultures tested, whether freshly isolated from natural substrates or taken from culture collections, were found to possess antagonistic properties. The selective antimicrobial activities of actinomycetes differ greatly, both quantitatively and qualitatively, as could easily be demonstrated by their respective antibiotic spectra. The nature of the active agents or the antibiotics produced by these organisms depends upon the species; frequently upon the strain; the composition of the medium in which it is grown, and the conditions of cultivation.[7, 8]

Several distinct antibiotics have now been isolated from cultures of actinomycetes. Some, namely, actinomycetin, micromonosporin, mycetin, and actinomyces lysozyme, have been only partly purified, whereas others, including actinomycin, proactinomycin, streptothricin, and streptomycin, have been isolated and crystallized. These substances differ greatly in their chemical structure, antimicrobial properties, toxicity to animals, and *in vivo* activity.

Some of the antibiotics are produced in simple synthetic media; others are formed in complex organic substrates; still others, like streptomycin, require the presence in the medium of a specific nutritive substance, an "activity factor," which is either a precursor or a prosthetic group of an enzyme system essential for the production of the antibiotic agent. Although this "activity factor" can be synthesized by *Streptomyces griseus*, its addition to the medium favors the rapid production of streptomycin. *Streptomyces griseus* can, therefore, grow

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* Partly supported by a grant from The Commonwealth Fund of New York.

† Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

well in substrates lacking the above factor, but the production amount of streptomycin is slower. However, when the mycelium thus formed is used as a constituent of fresh medium, streptomycin will be produced satisfactorily.

The following investigations were undertaken for the purpose of elucidating several important problems pertaining to the production of antibiotic substances by actinomycetes, namely: (1) the use of selective antibacterial spectra, for the purpose of establishing the formation of antibiotic type compounds by various species or strains; (2) the ability of different organisms to produce the same antibiotic substance; (3) the variation in chemical nature and in biological properties of a given antibiotic substance, formed by different organisms or under different conditions of culture.

EXPERIMENTAL

Methods

A large number of cultures of actinomycetes were isolated from differently treated soils, composts, and other substrates, and were tested for their antibiotic potency against several test bacteria. For isolation purposes, either ordinary synthetic media (glucose-asparagine agar) or organic media (nutrient agar, peptone-glucose agar) were used. In some cases, the following special selective media were also employed:

1. TB agar, consisting of 1.5 per cent washed agar, 0.05 per cent KH_2PO_4, 1 per cent glucose, and washed viable cells of *Mycobacterium tuberculosis* H37, as the sole source of nitrogen. (Because of the difficulty of suspending the tubercle bacilli homogeneously throughout the medium, growth of the antagonist upon the small cell aggregates, rather than the production of a clear zone of lysis, was taken as the criterion of attack upon the tubercle organism.)
2. Bacterial agar, namely, washed mineral-glucose agar, as prepared above, to which centrifuged and washed cells of various gram-positive and gram-negative bacteria had been added.
3. Streptomycin-enriched agar, comprising nutrient agar to which had been added 5 to 25 mg. of purified streptomycin per ml. of medium.

Actinomycetes Active against Mycobacterium Species

The cultures of actinomycetes that were isolated from the TB agar were tested by the streak method. Nutrient agar and glucose asparagine agar were used, with *Mycobacterium phlei* as test organism.

This saprophyte grows rapidly and can be handled more easily than the pathogenic mycobacteria. The reason for adding the H37 tubercle bacillus to the washed agar medium was that this organism allows better growth of antagonistic microorganisms (bacteria, fungi, and actinomycetes) than *Mycobacterium phlei.*

TABLE 1

ANTAGONISTIC EFFECT OF DIFFERENT ACTINOMYCETES AGAINST *M. phlei*

Source of cultures	Number of cultures tested on		Activity by streak test							
			Zone of inhibition, mm.							
			0		1 to 3		4 to 8		9 to >9	
	NA*	GA*	NA	GA	NA	GA	NA	GA	NA	GA
Unenriched soil	12	13	4	1	1	..	1	5	6	7
Soil 1†	50	48	22	6	6	1	14	12	8	29
Soil 2‡	1	1	..	..	..	..	..	1	1	..
Soil 3††	34	34	10	4	4	3	12	4	8	23
Straw compost	12	12	3	2	3	..	4	5	2	5
Stable manure	2	2	1	1	..	..	1	1	..	..
Leaf compost	9	8	2	..	1	1	2	1	4	6
Total cultures	120	118	42	14	15	5	34	29	29	70
Per cent of cultures	..	..	35	12	13	4	28	25	24	59

* NA=nutrient agar; GA=glucose asparagine agar.
† Enriched with washed viable TB cells.
‡ Enriched with washed viable cells of gram-negative bacteria.
†† Enriched with dead TB cells.

Those actinomycetes that were found by the streak method to be most active against *Mycobacterium phlei* were then grown in nutrient broth and in glucose-asparagine media containing 0.25 per cent agar, in order to obtain better surface pellicle formation. After incubation for 8 days at 28° C, the filtrates were sterilized, by heating at 70° C for 10 minutes, and tested. The results presented in TABLE 1 show that more actinomycetes were antagonistic to *Mycobacterium phlei* when tested on glucose-asparagine agar, than on nutrient agar. With the synthetic medium, twelve per cent of the cultures exhibited no antagonistic activity by the streak test, as compared with 35 per cent inactive cultures on the organic medium. The highest activity, namely 9 to > 9 mm. zone of inhibition, was shown by 59 per cent of the cultures on the synthetic, and by only 24 per cent on the organic, medium. When these cultures were grown in liquid or in semi-solid agar media, and the filtrates tested, the reverse was found to be the case, the organic broth proving to be a better medium for the production of antibiotic substances than the synthetic medium. This may be due to the larger amount of growth on the organic medium. The percentage of actinomycetes active against *Mycobacterium phlei* was fairly high and was well correlated with the distribution of antagonistic actinomycetes previously reported for the gram-positive and gram-negative bacteria.[4, 6]

The enrichment of the soil with living or dead TB cells appeared to exert no appreciable stimulation upon the development of antagonists, as compared with the control soil. Although the enriched soils showed a marked stimulation of the total number of actinomycetes, these organisms did not necessarily possess antagonistic properties.[9] Many of the strains developing on the TB-washed agar plate, to the extent of about 20 per cent of the total number, were found to belong to the *Streptomyces coelicolor* group, which comprises organisms not antibacterial in nature.

Actinomycetes Active against Gram-Negative and Gram-Positive Bacteria

A number of actinomycetes were picked at random from plates prepared from various natural materials. These cultures were tested, by the streak method, against *Escherichia coli* and *Bacillus subtilis*. The results presented in TABLE 2 show that, as a rule, a much larger proportion of the cultures were active against *Bacillus subtilis* than against *Escherichia coli*. Some of the cultures had about equal activity against both organisms, and one had greater activity against the gram-negative, than against the gram-positive, test-organism.

Just as in the case of the actinomycetes active against *Mycobacterium phlei*, more organisms displayed antagonistic action, by the streak method, on glucose-asparagine agar than on the nutrient agar. As compared to the 85 per cent of the cultures that showed lack of activity against *Escherichia coli*, when tested on the asparagine agar, 93 per cent of the cultures were inactive, when grown on nutrient agar; the corresponding percentages showing no activity against *Bacillus subtilis* were 41 and 64 per cent. The cultures giving the highest activity against *E. coli* were 6 and 3 per cent, and against *B. subtilis*, 44 and 16 per cent, when tested on glucose-asparagine and nutrient agar, respectively. The reverse was true, when the culture filtrates were tested,

TABLE 2

ANTAGONISTIC EFFECT OF DIFFERENT ACTINOMYCETES AGAINST *E. coli* AND *B. subtilis*

Source of cultures	Number of cultures tested	Zone of inhibition, mm.											
		E. coli						*B. subtilis*					
		0		1 to 3		4 to 9		0		1 to 3		4 to 9	
		NA*	GA	NA	GA	NA	GA	NA	GA	NA	GA	NA	GA
Control soil	18	16	11	2	4	..	3	12	4	4	2	2	12
Chicken throat	21	16	16	3	2	2	3	12	9	3	4	6	8
Soil No. 2*	41	38	36	2	3	1	2	17	11	16	4	8	26
Field Soil 17A (no stable manure)	12	11	11	..	1	1	..	6	5	1	1	5	6
Field Soil 18A (received stable manure)	22	21	17	..	3	1	2	14	8	6	1	2	13
Leaf and twig compost	37	36	33	1	3	..	1	32	22	..	11	5	4
Straw compost	14	14	14	..	..	..	..	11	6	2	1	1	7
Stable manure	22	22	20	..	1	..	1	16	12	6	5	..	5
Total	187	174	158	8	17	5	12	120	77	38	29	29	81
Per cent		93	85	4	9	3	6	64	41	20	16	16	44

* See footnote for TABLE 1.

nutrient broth proving to be a better medium than glucose-asparagine broth for the production of the antibiotic agents.

The results obtained for the soil enriched with washed viable cells of gram-negative bacteria show that such treatment did not bring about any significant stimulation of actinomycetes antagonistic to either *Escherichia coli* or to *Bacillus subtilis,* as compared with the unenriched control soil. This confirms the results reported elsewhere,[9] concerning the doubtful significance of the soil enrichment procedure for the isolation of specific antagonistic organisms.

Ninety-one cultures were selected at random and tested for their antagonistic properties against various bacteria, by the agar streak method. One or more of the following three media were used: nutrient agar, glucose-peptone agar, and glucose-asparagine agar. Of these cultures, 47 showed some activity against either, or both, of the gram-negative bacteria, on one or more of the above media. The distribution of the antibiotic activity of the cultures against the two bacteria is brought out in TABLE 3. In general, there were more actinomycetes active, in the case of all three media, against *Pseudomonas aeruginosa* alone, than against *Escherichia coli* alone or against both bacteria. Glucose-asparagine agar gave zones of bacterial inhibition which were wider than those observed on the organic media. The interesting phenomenon, in this connection, is that *Pseudomonas aeruginosa,* an organism which is usually recognized as highly resistant to most of the known antibiotic agents, was found to be more sensitive, at least as measured by the streak method, to the action of antagonistic actinomycetes than the ordinarily more sensitive *Escherichia coli.* Whether

TABLE 3

ACTINOMYCETES ACTIVE AGAINST *Escherichia coli* AND *Pseudomonas aeruginosa*

Test medium	Total number of cultures tested	Number of cultures active against:			
		E. coli only	*Ps. aeruginosa* only	*E. coli* and *Ps. aeruginosa*	Neither organism
Nutrient agar	36	7	12	6	11
Glucose-asparagine agar	40	5	14	8	13
Fungus agar (pH 7.0)	34	8	10	3	13

this is due to the formation by these actinomycetes of as yet unknown substances active against *Pseudomonas aeruginosa,* or whether the presence of the cells of the antagonist influences, in some manner, the sensitivity of *Pseudomonas aeruginosa,* still remains to be determined. The fact that *Streptomyces lavendulae* showed, by the streak method, some activity against *Bacillus mycoides,* whereas the antibiotic streptothricin produced by *S. lavendulae* is inactive against this bacterium, may also be due to factors still unrecognized, at present.

A detailed study was next made of the antagonistic properties of the actinomycetes that had been isolated from the washed agar media enriched with *Escherichia coli* and *Bacillus subtilis* cells. In all, 44 cultures were tested by the streak method, using three different media and four test bacteria. The results (TABLE 4) show that greater inhibition was exhibited by the actinomycetes on all the media against gram-positive, than against gram-negative, bacteria. There were marked differences, however, in the sensitivity of the different bacteria within each group. When the two aerobic spore formers were compared, *Bacillus mycoides* was found to be more resistant than *Bacillus subtilis* to most of the actinomycetes, both on nutrient agar and on glucose-tryptone agar. On the synthetic medium, however, the two spore formers exhibited very little difference in sensitivity to the various actinomycetes. A more detailed examination was made of the behavior of these two test organisms toward a number of actinomycetes. These results can be only briefly summarized here. In the case of nutrient agar, 20 cultures were more active against *Bacillus subtilis* than *Bacillus mycoides;* 7 were equally active against both bacteria; and 3 were more active against *B. mycoides* than against *B. subtilis.* The corresponding ratios of sensitivity of the two bacteria, to various actinomycetes grown on glucose-asparagine agar, were 9:20:9 (greater:

TABLE 4

ANTAGONISTIC ACTION OF ACTINOMYCETES ISOLATED FROM *E. coli-* AND *B. subtilis-* WASHED AGAR PLATES*

Test organism	Nutrient			Glucose-asparagine				Glucose-tryptone		
	0	1–8	9–16	0	1–8	9–16	>16	0	1–8	9–16
A. aerogenes	30	14	0	18	15	5	6	35	8	0
E. coli	21	22	1	5	22	4	13	27	15	1
B. mycoides	24	17	3	2	15	5	22	8	32	3
B. subtilis	15	23	6	3	13	8	20	4	33	6

* Forty-four cultures of actinomycetes were tested on nutrient agar and glucose-asparagine agar; 43 cultures, on glucose-tryptone agar.

equal:lower activity against *Bacillus subtilis*), and on glucose-tryptone agar, 22:6:10.

A comparison of the sensitivity of the two gram-negative bacteria brought out the fact that, on all the media used, *Aerobacter aerogenes* was more resistant to the action of actinomycetes than was *Escherichia coli.* But here, as well, a more detailed examination of the plates revealed certain striking differences: on nutrient agar, 20 cultures were more active against *Escherichia coli* than against *Aerobacter aerogenes,* 2 were active alike against both bacteria, and 3 were more active against *A. aerogenes* than against *E. coli.* The corresponding ratios for the sensitivity of the two bacteria on the other two media were 35:1:2 and 14:0:2.

Antibiotic Properties of *S. griseus*-like Organisms

Streptomycin is the most important antibiotic so far obtained from actinomycetes. It was found to be produced by two strains of *Streptomyces griseus* isolated, in 1943, from two distinct sources. The original culture of *Streptomyces griseus,* which has been kept in the culture collection since 1915, does not possess now the property of producing streptomycin, and, when recently tested, was found to exert only a very weak antibiotic effect against *Bacillus subtilis* and none against *Escherichia coli.*

In order to determine whether the capacity to form streptomycin is characteristic only of freshly isolated strains of *Streptomyces griseus,* and how widely distributed this capacity is among members of the *S. griseus* group of actinomycetes, a number of cultures, recognized by macroscopic appearance to be typical *S. griseus,* or to be forms closely resembling it, were isolated from different substrates. These cultures were first tested for antibiotic activity by the agar streak method, using nutrient agar and glucose-tryptone agar. Streptomycin is produced largely on the first medium and only to a limited extent on the second. The results presented in TABLE 5 show that the various *Streptomyces griseus*-like cultures thus isolated were far more active against gram-positive, than gram-negative, bacteria. They showed greater activity against gram-negative bacteria on nutrient agar, than on glucose-tryptone agar. Even that activity, however, was limited in nature, as compared with the streptomycin-producing strains of *Streptomyces griseus.* When grown in liquid media, none of the freshly isolated *Streptomyces griseus* strains produced the typical streptomycin. Only one culture, namely, 25-G, was capable of yielding an active filtrate, as shown in TABLE 6. The anti-

TABLE 5

DISTRIBUTION OF ANTAGONISTIC PROPERTIES AMONG ACTINOMYCETES BELONGING TO THE *Streptomyces griseus* GROUP

Test organism	Zone of inhibition, mm.							
	Nutrient agar*				Glucose-tryptone agar†			
	0	1–3	4–8	9–>15	0	1–3	4–8	9–>15
A. aerogenes	17	9	4	0	17	6	1	2
E. coli	1	9	17	3	13	8	2	3
B. mycoides	2	11	12	5	1	10	9	6
B. subtilis	1	6	18	5	1	5	12	8

* 30 cultures tested.
† 26 cultures tested.

TABLE 6

BACTERIOSTATIC SPECTRA OF 3 DIFFERENT PREPARATIONS FROM ACTINOMYCETES

Culture No.	Units of activity per ml. of crude culture filtrate against:			
	E. coli	*B. subtilis*	*B. mycoides*	*S. aureus*
3440	50	300	10	50
3445	150	300	20	150
25-G	100	130	10	250

biotic nature of this filtrate appeared to be different from both streptothricin and streptomycin, as shown by its antimicrobial spectrum.

These results prove the principle that the production of a given antibiotic by actinomycetes is characteristic of the strain of organism, rather than of the species.

General Considerations of Antimicrobial Properties of Actinomycetes

As a result of a comprehensive study of the antimicrobial properties of a large number of cultures of actinomycetes isolated from different sources, and tested on the various media listed above, the general types of activity can be summarized as follows:

1. Certain cultures of actinomycetes are largely active against fungi, but not at all, or only to a limited extent, against bacteria.
2. Some cultures are active, primarily, against other actinomycetes, and only to a limited extent against bacteria.
3. A large number of actinomycetes affect mycobacteria, notably *Mycobacterium phlei,* but not at all, or only to a limited extent, various other bacteria.
4. Many actinomycetes are active against gram-positive bacteria and mycobacteria, but not against gram-negative bacteria.

5. Various actinomycetes are active against both gram-positive and gram-negative bacteria, with certain marked variations, namely:

(a) some are more active against gram-positive bacteria;
(b) some are more active against gram-negative bacteria;
(c) some are equally active against both groups of bacteria.

The antimicrobial properties of a given actinomyces culture also depend upon the composition of the medium in which it is grown. One organism is equally active against *Escherichia coli* and *Aerobacter aerogenes* on nutrient agar, whereas it inhibits only *E. coli* on glucose-asparagine or glucose-tryptone media. Another culture is equally active against *Bacillus mycoides* and *Bacillus subtilis,* on nutrient agar only. A third culture may produce equal inhibition on all three media, against the two gram-positive bacteria. A fourth culture, which is more active against *Bacillus subtilis* on glucose-asparagine agar, may be more active against *Bacillus mycoides* on nutrient agar.

6. Finally, there is a group of properties characteristic of certain actinomycetes, namely, activity against bacteriophages; this activity may or may not extend to the host bacteria.

Production of Antibiotic Substances by Actinomycetes

Some of the specific antimicrobial agents or antibiotics produced by actinomycetes have been isolated from suitable cultures of the organisms, either in a crude or in a pure state. A much larger number of substances still remains to be isolated, however, since it can be established beyond any doubt, by testing of the crude filtrates, that such substances exist. Among those agents that have not as yet been isolated, but the formation of which is unquestioned, the following may be given here only brief consideration:

1. *Substances active against Mycobacterium phlei, but not against gram-positive or gram-negative eubacteria.* A certain strain of *Streptomyces lavendulae* (No. 3465), grown in glucose tryptone medium, was found to produce in stationary culture, after 6 and 11 days' incubation, a factor which was heat stable and which inhibited *Mycobacterium phlei* in a dilution of 1:300. This filtrate had no activity against *Bacillus subtilis, Bacillus mycoides, Escherichia coli,* and *Pseudomonas aeruginosa.* The same observation was made in the case of another culture, which was also found to belong to the *Streptomyces* group.

2. *Substances active against bacteriophages.* Certain cultures of actinomycetes have been found to produce factors present in the culture

filtrates, which were devoid or nearly devoid of antibacterial properties, at least in the concentration tested, yet which inhibited the lysis of bacteria by phages. Whether the action of these factors is directed against the phage particles or against the host cells, resulting in the alternation of their metabolism so as to prevent phage infection or multiplication, still remains to be determined. The production of these antiphage filtrates is dependent upon the composition of the medium, in a manner similar to the production of true antibiotics. The antiphage action of the filtrates is also specific, some phages being affected, and not others. This selectivity is comparable to that of the selective antibacterial action of known antibiotics.

3. *Agents active against animal viruses.* Certain culture filtrates of various actinomycetes appear capable of exerting a slight, but definite, inactivating effect upon animal viruses. In one case, the filtrate was not antibacterial, although test bacteria were found to be inhibited on certain media, as measured by the streak method. From the active filtrate of another culture, actinomycin was isolated, as the virus-inactivating agent.

4. *Antibiotics active against bacteria and fungi.* Although several antibiotics have already been isolated from actinomycetes, still others remain to be obtained. Those isolated, so far, vary greatly in their chemical nature and in their selective antimicrobial action.

Among the problems bearing upon the isolation of antibiotic substances, two deserve special consideration:

(a) It is now well established that certain actinomycetes produce more than one antibiotic substance. *Streptomyces griseus* produces streptomycin and another, still undescribed factor, present in the mycelium of the organism. This factor is soluble in organic solvents and differs markedly, in its antibacterial spectrum, from streptomycin.

(b) The same antibiotic may be produced by different species of actinomycetes. Certain unpublished data from our laboratory have established, beyond doubt, that actinomycin is produced by several species of *Streptomyces*. The same is true of streptothricin. A given antibiotic may, therefore, be identical, even when produced by different actinomycetes, as shown by its chemical composition and antibiotic spectrum. In the case of other antibiotics, however, certain differences in the specific antibacterial spectra of the same type of substance produced by different organisms appear to point to differences in the chemical structure of the antibiotic. The present information is too limited, however, to justify broad generalizations. However, it opens some interesting possibilities.

SUMMARY AND CONCLUSIONS

The results of a survey of a large number of actinomycetes, isolated from different substrates and cultured on different media, permit certain broad generalizations:

1. A large proportion of the actinomycetes inhabiting natural substrates have the capacity to inhibit the growth of bacteria and other microorganisms.

2. The ability of these actinomycetes to exert an inhibiting effect upon microorganisms is highly specific. This selectivity depends, not only on the strain of organism, but also on the medium in which it is grown and conditions of growth.

3. Antagonistic actinomycetes produce a variety of antibiotics that vary in chemical nature, in antimicrobial action, in toxicity to animals, and in their chemotherapeutic potentialities.

4. The antibiotics that have, so far, been isolated from actinomycetes vary in the degree of purification. Some are crude preparations, whereas others have been crystallized, and considerable information has been gained concerning their chemical nature. They include: actinomycetin, actinomyces lysozyme, actinomycin, micromonosporin, streptothricin, streptomycin, and mycetin.

5. Some actinomycetes produce more than one antibiotic substance.

6. Some antibiotics are produced by several different organisms. There is some evidence, however, that, although the same general type of substance may be formed by the various cultures, it may not possess exactly the same antibiotic spectrum; this suggests the possibility of variations in the chemical structure of the agent.

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ANTIBIOTIC SUBSTANCES PRODUCED BY BACTERIA

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In 1877, Pasteur[1] reported that laboratory animals, injected with the causative agent of anthrax (*Bacillus anthracis*) and common saprophytic bacteria, failed to develop the disease. This effect was ascribed to oxygen-starvation of the anthrax organism, brought about by utilization of oxygen by the saprophyte. During the years that followed, the inhibitory action was found to be associated with many mixed cultures and has come to be known as microbial antagonism. We now know that antagonisms, in the majority of cases, may be charged to the production by one microorganism of metabolic products which have toxic or inhibitory activity for other microorganisms. Such chemical identities have come to be known as antibiotics.

As is often the case with scientific studies, chance observation led to the study of many antibiotics. Thus, Emmerich,[2] during a demonstration before his class, accidentally discovered that a guinea pig that had been previously injected with a culture of *Streptococcus erysipelatis* did not develop cholera when inoculated with *Vibrio cholerae*. This was followed closely by Bouchard's[3] observation that rabbits, previously inoculated with *Bacillus anthracis,* did not develop anthrax when small amounts of cultures of *Pseudomonas aeruginosa* were injected, a finding that was later extended to include sterilized *Pseudomonas aeruginosa* cultures. The mass of literature, often conflicting, that has accumulated concerning these antagonistic activities, leads one to realize that only through the chemical isolation of the antibiotics concerned is it possible to completely understand the antagonistic phenomena. In the case of *Pseudomonas aeruginosa* antagonisms, the activity has been variously ascribed to enzymes, fatty acids, surface tension depressants, pigments, etc. In the 67 years since this type of antagonism was first noticed, no less than two phenazine derivatives, with their structure confirmed by synthesis, and five crystalline compounds of unknown structure, all with antibiotic activity, have been isolated from this one organism. Is it any wonder that different investigators, working with different strains of this organism and different media, have reported conflicting results?

Returning to the discovery of antagonistic bacteria, four conditions have furthered their investigation. The first of these is chance, which is materially aided by the investigator's curiosity and perception. Secondly, comes natural indication of antibiotic action. Active antagonists, such as many of the pseudomonads, exert a directing effect upon natural bacterial populations. Thus, the typhoid organism and even *Escherichia coli* do not persist in water basins where conditions are favorable for development of *Pseudomonas fluorescens*. Promoting development of spore-forming bacteria in soil, with green manures, or favoring development of the fungus *Trichoderma* in soil, materially reduce attack of the damping-off fungi on seedlings. Such natural phenomena have led to the discovery of antagonists. Thirdly, distinctive characteristics, such as color, insolubility in culture medium, etc., have led to the isolation of compounds which later proved to be antibiotics. Fourthly, antibiotics have been obtained by planned experimentation. The early success of Dubos,[4] in applying the enrichment procedures, as used in soil microbiology, to the isolation of bacteria producing enzymes specifically lytic for the capsular polysaccharides of the pneumococci, has been described often. In the presence of a resistant food source, those microorganisms possessing the specific enzyme for that food will find conditions favorable for development. Those bacteria possessing the specific enzyme, or capable of producing an active adaptive enzyme, will multiply. However, the enrichment procedure is not directly applicable to the isolation of antagonists. Essentially, the method requires the addition of living microorganisms to the soil. It has been suggested that antagonists in the soil destroy the added bacteria by the production of antibiotics, and then multiply at the expense of the killed bacteria.

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Destruction of foreign organisms, such as *Escherichia coli* and *Mycobacterium tuberculosis,* by biological means in a bacteria-rich substrate, such as soil, has been proved (TABLE 1). The biological destruction became more efficient, as new additions of the foreign organism were made. Each enrichment consisted of the addition of billions of living *Escherichia coli* cells per gram of soil. After eleven enrichments, less than 1000 *Escherichia coli* cells were recovered per gram of soil, whereas more than six billion were present per gram following the first enrichment. After the soil had been freed of living microorganisms by heat sterilization, *Escherichia coli* multiplied.

In one sense, this is the equivalent of the enrichment method for the isolation of cellulose decomposers, or organisms active against the pneumococcus polysaccharide, since the enrichment culture became more active with enrichment. However, it has not been possible to show an increase in the proportion of antagonists specifically associated with

TABLE 1

SURVIVAL OF *Escherichia coli* IN STERILE AND NON-STERILE SOIL
Thousands per gram

Sterile soil		*Fresh soil*			
				No. of microorganisms	
Incubation period days	No. of *E. coli*	Incubation period days	*E. coli* enrichments*	*E. coli*	Total population
0	2.6†	0	0	0	.....
10	149,000	5	1	6,800	25,600
26	138,000	33	5	130	22,100
..	....	127	11	0	40,000

* Approximately one week elapsed between the last enrichment and the determination of surviving *Escherichia coli.*
† 2,600 cells of *Escherichia coli* added per gram of sterile soil.

TABLE 2

INFLUENCE OF ENRICHMENT OF SOIL WITH *Escherichia coli* ON THE ABUNDANCE OF ANTAGONISTS

Number of days	Number of microorganisms (thousands)		Number of antagonists* (thousands)		Antagonists %	
	Control	Enriched	Control	Enriched	Control	Enriched
37†	8,800	46,000	500	4,000	5.7	8.7
127‡	9,100	40,000	1,150	5,700	12.6	14.2

* Microorganisms having a lytic action on living *Escherichia coli* cells in the absence of organic nutrients.
† Five enrichments before the first count.
‡ Eleven enrichments before the second count.

the living enrichment agent, in such enrichment cultures (TABLE 2). Although numbers of antagonists did increase with enrichment, this increase was proportional to the increase in total bacterial population. Similar results could have been obtained by the addition of any nutrient source to the soil, and are not specific for the addition of living bacteria. The chances of random selection of an antagonist from an enriched soil are not better than from a control soil.

By adding nutrients, it may be possible to modify the soil population, so that one group (for example, the spore formers) predominates, and in this manner, change the inherent antagonistic power of the soil. Such changes, however, are not specific, or limited to those produced by enrichment with living bacteria. For practical isolation purposes, the enrichment cultures must be studied by means of some selective method that will not only allow detection of the increased number of antagonists, in the enrichment culture, but will eliminate the increased number of non-antagonistic bacteria. This is relatively simple when antagonists possessing lytic activity are desired, since antagonistic colonies on agar plates containing a dense suspension of bacteria will be surrounded by zones of lysis. No satisfactory selective medium that will allow growth of antagonists showing only bacteriostatic or bactericidal, but not lytic, activity has yet been devised.

It is evident, from the studies of Stokes and Woodward,[5] that enrichment of soil with gram-positive cocci was not necessary for the discovery of the bacillus that produces tyrothricin. Similar antagonists, producing tyrothricin, are present in every soil. They may be easily demonstrated by plating soils in a rich organic medium at low concentration. Subsequent crowding serves to demonstrate antagonists. Colonies surrounded by clear zones where spreaders are inhibited may be isolated as possible antagonists.

Of 24 antagonists picked from plates similar to these, seven proved to be characteristic tyrothricin-producing strains, all similar in biochemical characteristics. These proteolytic spore-forming rods are normal inhabitants of soil and form the zymogenic population of the soil. As such, they exist in normal soils only as resting or slowly dividing cells, becoming active in the presence of easily-decomposable, nitrogenous organics. It is of little importance to the spore-forming antagonist whether this food is present in the form of peptone, leaves, or *Escherichia coli* cells.

Thus, despite its successes, the planned development of microbial antagonists is a useful tool only when a complete understanding is had of the processes involved.

No attempt can be made here to review completely all bacterial antibiotics. Rather, it will be more practical to examine a group of antagonistic bacteria of promising economic and theoretical interest. The spore-forming bacteria meet these qualifications, because of recent successes in detoxification, treatment of experimental infections, and suggestions of mode of action. Whereas any discussion of spore-forming antagonists must begin with gramicidin, it is only with the most recent work that we need be concerned.

Gramicidin and tyrocidine hydrochloride, isolated in crystalline form and studied chemically by Dubos[4] and Hotchkiss,[6] and gramicidin S, obtained crystalline by Gause and Brazhnikova,[7] all are obtained from *Bacillus brevis*. Being polypeptides, poorly soluble in water, but soluble in alcohol, these crystalline antibiotics may be differentiated with certainty, on the basis of chemical characteristics alone. They are unusual natural compounds, since a portion of the amino acids liberated upon hydrolysis are of the unnatural form (TABLE 3).

TABLE 3

(From Belozersky, A. N., & T. S. Passhina. Lancet 247(2): 716–717. 1944).

	Gramicidin Dubos	Tyrocidine hydrochloride	Gramicidin S
Melting point	228–230°C.	240°C.	268–270°C.
Molecular weight (Rast)	1250–1550	1260	1060–1340
Free NH_2	–	+	+
Free COOH	–	+	+
Time of dissolution on hydrolysis (hours)	18	2	18–20
Total N (Kjeldahl)	14.8%	14.3%	13.0%
Tryptophan	+	+	–
Tyrosine	–	+	–
Dicarbonic acids	–	+	–
Phenyl-alanine	–	+	–
Proline	–	+	+
Ornithine	–	+	+
Leucine	+	+	+

The relative clinical value of the three preparations is not as well established. While of value in the treatment of local infections, the toxicity has prevented wider application for systemic infections. Recently, Lewis *et al.*[8] have been able to detoxify gramicidin, without altering antibacterial activity, by treatment with formaldehyde. Clinical studies with detoxified gramicidin have not been reported.

The mode of action of the antibiotics from *Bacillus brevis* has been explained upon the basis of change in surface tension brought about by gramicidin and tyrocidine. Stimulation of oxygen uptake, in studies with *Staphylococcus aureus,* have been noted. This phenomenon has led to a suggestion that gramidicin blocks synthesis of polysaccharide storage products by the cell.

Gramicidin blocks phosphate uptake by cells, in concentrations which are bacteriostatically effective. The quantity required for this effect is related to the sensitivity of the cell for gramicidin, and the action on phosphate uptake and bacteriostasis are both inhibited by cephalin (Hotchkiss[6]).

Characterization of the chemical properties of the gramicidins has aided materially in the isolation of a similar type of compound from *Bacillus subtilis.* This culture grows well and produces antibiotic activity on a wide variety of media, including both organic and inorganic types. As might be expected from this proteolytic species, polypeptides may be isolated from cultures. Jansen and Hirschmann[9] reported the isolation of a crystalline polypeptide by the gramicidin isolation procedure, but it was without antibiotic activity. The sediment obtained with a Sharples centrifuge, which contained the whole cells, was as active as isolated tyrothricin, and this active principle could be further purified ten times by ethanol extraction and acid precipitation of the antibiotic. Subtilin, as the substance was termed, proved to be highly diffusible, similar to penicillin, in contrast to the practically undiffusible gramicidin.

There is a possibility that subtilin may become useful as a therapeutic drug, since no reports of toxicity have appeared, and the range of organisms inhibited by it has been extended from the common gram-positive types to include *Mycobacterium tuberculosis.*

Bacitracin is an antibiotic of reported therapeutic activity. Published data concerning its chemical properties are meager. It has not been clearly differentiated from subtilin. Jern and Meleney[10] reported bacitracin concentrates of 100 units per cc. activity to be not as efficient as penicillin, in treatment of embryonic eggs infected with *Staphylococcus aureus,* but three out of four infected eggs survived for the experimental period. A later report by Johnson, Anker, and Meleney[11] demonstrated much greater sensitivity of hemolytic streptococci and clostridia for the antibiotic. *In vivo* action of bacitracin was demonstrated for both infections in animals. Thus, there is a promise of practical application for bacitracin.

Simplexin stands as an example of an antibiotic which is so poorly defined, chemically, as to be indistinguishable from other types, except for its characteristic antibacterial spectrum. In contrast to the more limited activity of the substances described previously, simplexin has been found active against 77 out of 81 species of parasitic and saprophytic fungi, as well as bacteria. First described by Cordon and Haenseler,[12] and later studied by Katznelson,[13] it has offered some promise of application against plant pathogens. Simplexin is remarkably stable. It may be adsorbed on charcoal and eluted with ethanol, but no additional information has been published concerning its chemical properties.

A new antibiotic, named bacillin, is included as a final example of antibiotic types from spore-forming bacteria.[14, 15] This substance, which differs from all antibiotics described from bacteria, is characterized by a remarkably constant bacteriostatic spectrum against a wide range of microorganisms. Bacillin may be differentiated clearly from subtilin and simplexin, on the basis of the bacteriostatic spectrum;

and from tyrothricin, on the basis of diffusibility, as well (TABLE 4). It is produced by *Bacillus subtilis* on synthetic media, and similarly to the formation of simplexin and subtilin, its formation is stimulated greatly by the presence of manganese salts in the medium. Bacillin may be concentrated by adsorption from the medium on charcoal (Darco) and elution with ethanol. Preparations obtained in this manner have inhibited growth of *Escherichia coli* in dextrose-asparagine agar in a concentration of 0.67 γ per ml. The presence of blood or organic media markedly reduces bacillin activity.

Table 4

Comparative Antibacterial Spectra of Antibiotics from Three Different Aerobic Spore Formers

Organism	Ratios of amount of antibiotic material required for inhibition*			
	E. coli as unity		*S. aureus* as unity	
	Bacillin	Simplexin	Bacillin	Subtilin
E. coli	1.0	1.0	2.7	>10
E. typhosa	0.5	1.0	1.4	>10
S. paratyphi	0.09	>32	0.25	10
Pasteurella sp.	0.37	0.13	...	...
D. pneumoniae	1.0	0.13	...	...
M. conglomeratus	...	...	2.0	1.0
S. aureus	0.37	>32	1.0	1.0

* Streak method on nutrient agar.

As expected from the scarcity of chemical information, little explanation can be given concerning the mechanism of action of most of the antibiotics from bacteria. Especially difficult is the explanation of wide range of inhibition from one species, or even strain, of test organism to another. Explanation of antibiotic action of bacillin need not be concerned with this point.

The neutralizing effect of para-aminabenzoic acid upon the antibacterial activity of sulfanilamide and derivatives has led to the synthesis and demonstration of antibacterial activity of a number of compounds related to growth-promoting compounds. A similar mode of action has been suggested by McIlwain[16] for iodinin, the antibiotic of *Chromobacterium iodinum*. Iodinin is antagonized by substances having vitamin K activity. Four possibilities were considered by McIlwain: (1) reaction between inhibitor and antagonist, in the absence of the organism; (2) interaction, in the presence of the organism; (3) action of substances that normally promote growth of the organism; and (4) other cases, in which the antagonist is more specifically related to the process of inhibition. Statement (2) applied to iodinin antagonism (TABLE 5). A competitive mechanism was proposed for the interaction. Competition existed between the antibiotic iodinin and certain non-antibiotic quinones. Cells that adsorbed the latter were capable of multiplying. Then, the iodinin was detoxified by reduction.

The action of cephalin upon gramicidin is not similar, because of the dissimilarity of these compounds. Proof of a competitive action has not been reported for other bacterial antibiotics.

The discovery that bacillin had reduced activity in complex organic media led to the investigation of the factors responsible (TABLE 6). It

TABLE 5

MUTUALLY NEUTRALIZING QUANTITIES OF IODININ AND 2-METHYL-1:4-NAPHTHAQUINONE
(From McIlwain, H. Biochem. J. 37: 265–271. 1943.)

Iodinin (M)	Time (days) before appearance of visible growth in medium containing 2-methylnaphthaquinone (M)					
	4×10^{-6}	2×10^{-6}	1×10^{-6}	5×10^{-7}	2.5×10^{-7}	1.2×10^{-7}
2×10^{-5}	1¼	2	>7	>7	....	....
1×10^{-5}	¾	2	3	>7	....	....
5×10^{-6}	...	1¼	1¼; 2	3; 7	....	....
2.5×10^{-6}	...	¾	¾; 1¼	1¼; 2	>7	....
1.2×10^{-6}	...	¾	¾	¾; 1¼	>7	>7
6×10^{-7}	...	...	...	¾	3	>7
3×10^{-7} and less	...	...	...	¾	¾	¾

TABLE 6

INHIBITORY EFFECT OF COMPLEX ORGANIC MATERIALS OF BACILLIN ACTIVITY
(Dextrose-asparagine medium)

Substance added	Amount per ml. agar	Dilution of bacillin concentrate inhibiting *E. coli*
None	......	24,000–48,000
Brain-heart infusion, Difco	10 mg.	375–750
N-Z-Case, Sheffield Farms	10 mg.	375–750
Tryptone, Difco	10 mg.	3,000–6,000
Yeast extract, Difco	10 mg.	6,000–12,000
Beef extract, Difco	10 mg.	12,000–24,000
Liver extract, Wilson	10 mg.	12,000–24,000
Beef blood, Difco	10 mg.	24,000–48,000
Fresh whole rabbit blood	0.1 ml.	188–375
Fresh rabbit serum	0.1 ml.	1,500–3,000
Cephalin	1.0 mg.	24,000–48,000
NaCl	0.1 mg.	24,000–48,000
NaCl	1.0 mg.	24,000–48,000

may be noted that many of the common bacteriological complexes used for growth of bacteria had a depressing effect upon bacillin activity. Further, the depressing effect was found to be in direct relationship to the concentration of organic complex present in the medium.

Since the most effective inhibitors of the antibiotic activity were products that had undergone partial hydrolysis, further hydrolysis was accomplished with sulfuric acid (TABLE 7).

TABLE 7

EFFECT OF ACID HYDROLYSIS ON ANTIBACILLIN ACTIVITY OF NATURAL MATERIALS

Supplement to dextrose-asparagine agar	Dilution of bacillin concentrate inhibiting *E. coli* with	
	Unhydrolyzed supplement, 0.5 per cent	Hydrolyzed supplement, 0.125 per cent
None	7680–10,240	7680–10,240
Starch	7680	7680
Agar	7680	7680
Gelatin	5120–7680	480–640
Casein	>7680	640–980
Peptone	1920–2560	1280–1920
Beef extract	>7680	7680
Egg albumin	1280–1920	1280–1920
Wheat bran	>7680	5120–7680
Cottonseed meal	>7680	2840–5120
Milk (solids basis)	<320	1280–1920

Proteinaceous materials became more effective in neutralizing bacillin upon hydrolysis, and the two essentially pure proteins, gelatin and casein, were highly effective after hydrolysis, although of no effect before. Egg albumin and milk were the only unhydrolyzed materials having a neutralizing effect on the antibiotic. Because of a direct relationship existing between concentration of supplement and neutralization of antibiotic activity, the activity reduction reported in the hydrolyzed supplement column of TABLE 7 is one-quarter the amount that would have been obtained, if equivalent amounts of hydrolyzed and unhydrolyzed supplement had been tested.

Further studies with gelatin conclusively showed that the factor was liberated upon gelatin hydrolysis, and liberation was, in fact, dependent upon acid concentration (TABLE 8).

Liberation of the additional factor was found, with increased time of incubation at a constant acid concentration, but after an optimum period, the concentration of the factor decreased. Enzymes had a similar liberating effect.

It was possible to prove that the factor was organic in nature, remarkably stable and specific, and to purify it to the stage that 0.25 γ per ml. of agar would reduce the bacillin titer of a concentrate against *Escherichia coli* from 1:50,000 to 1:10,000. These facts indicated a compound of sufficient specificity for the name, antibacillin, to be applied to it.

TABLE 8

EFFECT OF STRENGTH OF ACID ON LIBERATION OF ANTIBACILLIN FROM GELATIN

Concentrate of H_2SO_4 used during hydrolysis[1]	Dilution of bacillin concentrate inhibiting *E. coli*[2]
No supplement	10,240
Unhydrolyzed gelatin	10,240
0.25N	2560–5120
0.5N	1280–2560
1N	320–640
2N	160
4N	160–320
6.7N	160–320

[1] For 1 hour at 120°.

[2] Concentration of hydrolysate=0.2 per cent in all cases. Dextrose-asparagine agar was the assay medium.

Consideration of the postulates of McIlwain, for explanation of neutralization of antibiotics, indicated that the antibacillin-bacillin effect is a competitive action, similar to that postulated for para-aminobenzoic acid-sulfanilamide or vitamin K-iodinin.

From mixtures of bacillin and antibacillin in which the antibiotic activity of bacillin is completely inhibited, both substances may be reisolated by a relatively mild adsorption procedure. The isolated substances are fully active. This is strong evidence for a competitive action (TABLE 9).

Attempts to demonstrate vitamin activity for antibacillin, in the same manner as previously established for para-aminobenzoic acid, have, so far, been inconclusive. Isolation as partial hydrolysis products from proteins suggests similarity to the strepogenin of Woolley and Sprince.[17] It is true that highly purified antibacillin preparations do have strepogenin activity. However, consideration of the relative strepogenin activity and antibacillin activity of a number of concentrates indicates a disparity that cannot be explained, if these two biologically active substances are considered identical (TABLE 10).

Demonstration of a vital function which antibacillin may play in the metabolism of the microorganism awaits further study.

TABLE 9

REISOLATION OF BACILLIN AND ANTIBACILLIN FROM MIXTURES

Active substance	*Bacillin activity** Dilution inhibiting *E. coli*		*Antibacillin activity** γ/cc. of agar to reduce bacillin activity to 1/10 of original	
	Before norite‡ process	After norite process	Before norite process	After norite process
Bacillin	51,200	19,200–25,600	....	....
Bacillin+antibacillin†	<1,600	12,800–19,200	....	60–120
Antibacillin†	....	....	60–120	60–120

* Dextrose-asparagine medium.
† Antibacillin prepared from hydrolyzed gelatin.
‡ Bacillin adsorbed from neutral solution on norite and eluted with 90% EtOH. Antibacillin not adsorbed.

TABLE 10

STREPOGENIN AND ANTIBACILLIN ACTIVITY OF DIFFERENT SUBSTANCES

Substance	Strepogenin activity*		Antibacillin activity†	
	mg./cc.	ratio	mg./cc.	ratio
Antibacillin concentrate	0.03	1.0	0.0067–0.013	1.0
Strepogenin concentrate	0.02	0.67	0.14–0.28	21
Hydrolyzed gelatin	10	330	1.0	100
Meat extract	0.22	7.3	8.3	830
Dried milk	4.4	150	0.10–0.20	15

* ½ maximum *L. casei* growth in synthetic medium.
† 90% reduction in *E. coli* units of bacillin in dextrose-asparagine agar.

In conclusion, it may be stated that the purpose of this discussion has not been to review knowledge of antibiotics from bacteria. Rather, it has been to point out types of studies needed for scientific appreciation of the field. It no longer does more than confuse matters merely to report inhibition of a group of organisms by a bacterial culture filtrate. A more exact chemical appreciation of the antibiotics concerned is needed. Exhaustive toxicity determinations and tests of therapeutic effectiveness upon these chemicals, then, become more significant. Finally, we are approaching the stage when evidences of mode of action of the antibiotics may be gathered into concrete theories.

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ANTIBIOTICS

PART II

PHARMACOLOGICAL

THE PHARMACOLOGY OF STREPTOTHRICIN AND STREPTOMYCIN

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Although streptothricin[1] and streptomycin[2] possess rather similar chemotherapeutic properties, they differ considerably in their toxicity and pharmacodynamic effects. Furthermore, since neither of them has, as yet, been synthesized, nor is available in absolutely pure form, all studies, to date, have been performed with preparations containing varying amounts of impurities. Many of the pharmacological properties observed may be due, therefore, to these, rather than to the active principles, a factor which obviously complicates the pharmacological and clinical evaluation.

It may seem inappropriate to open a discussion of the pharmacology of streptothricin and streptomycin by placing emphasis on impurities; yet, the longer one studies these drugs, the less can one escape the conviction that the pharmacology of streptomycin and streptothricin, in the form in which we know them today, is, to a very large degree, due to one of their "standard" impurities. I shall use this term, in the following discussion, to designate contaminants which, unless specifically removed, may be expected to be constantly present in preparations of the average type, varying only quantitatively, but not qualitatively, as long as the parent strains of *Aspergillus lavendulae* and *Aspergillus griseus*, the composition of the nutrient media, and the processes of extraction, concentration, and finishing, remain the same. In contrast to these "standard" impurities, which are as much a part of the average streptomycin and streptothricin preparation as the active principle itself (but which vary widely with preparations of different origin), there may be found non-specific impurities, resulting from the use of impure ingredients, accidental contamination, errors in manufacturing, improperly cleaned equipment or containers, etc. We shall not concern ourselves with this type of non-specific impurity, which is rare and should never be encountered in a preparation that has been released for clinical use, provided, of course, that a rigid system of control is maintained.

The existence of "standard" impurities, in practically every preparation of streptothricin and streptomycin, needs to be stressed, because some statements, though based on valid experimental evidence, may later have to be modified or completely discarded, once the experiments have been repeated with absolutely pure material. Nevertheless, the presence of these impurities does not significantly alter the results of the bacteriological potency assay and, for this reason, has probably no direct bearing on the chemotherapeutic usefulness of these drugs, except that it may cause, or aggravate, some undesirable side-effects.

In order to present as clearly as possible the complex picture of the pharmacology of agents which regularly carry varying amounts of several pharmacodynamically highly active impurities, I should like to describe, first, the findings obtained with average preparations of streptothricin and streptomycin, such as are generally used in the clinic. Next, we shall examine to what extent these pharmacological properties are modified by the "standard" impurities. Lastly, we shall review the possible clinical significance of each "standard" contaminant and, in this connection, discuss some of the presently adopted official standards. These are entirely based on pharmacological findings and represent a compromise between the desire to provide a safe agent, of known chemotherapeutic potency, and as little interference as possible with the production of this urgently needed new drug which, at this time, cannot be produced economically, in pure form, on a commercial scale.

The use of impure drugs in human therapy is, of course, nothing new or unusual. Patients have benefited for centuries from the prescription of such remedies as digitalis and ergot, and more recently, extracts of various glands, vitamin concentrates, protein hydrolysates, etc. As long as the amount and type of impurity are known and controlled, there can be little objection to the use of impure drugs, which indeed represent a major portion of today's therapeutic armament.

Streptothricin and streptomycin, which, in their particular domain, rival the therapeutic efficacy of penicillin, do not, however, share its almost complete pharmacodynamic inertness. Streptothricin is by far the more toxic of the two, but streptomycin, likewise, possesses, even in highly purified form, toxic properties that place a definite upper limit on the doses which can safely be given.

When injected into mice or rats, both agents produce quite similar effects. Almost immediately after an intravenous injection and only a few minutes after a subcutaneous injection, the animals exhibit respiratory difficulties, lose consciousness and, with sufficiently large doses, may die from asphyxia, the heart continuing to beat for several minutes after complete cessation of breathing (FIGURE 1). However, while animals which recover from the first impact of a sublethal dose of strep-

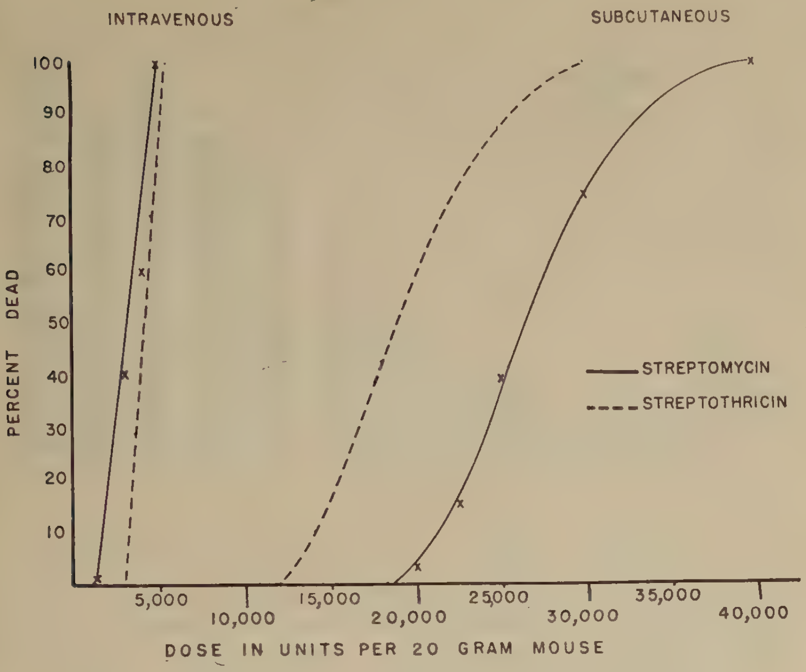

FIGURE 1. Comparison of acute toxicity of streptomycin and streptothricin.

tomycin survive without further ill effects,[3, 4] animals injected with streptothricin, after 1–2 days of apparently complete well-being, lose their appetite. Their fur becomes ruffled, they breathe with increasing difficulty, the urinary output decreases, and they die within several days after reappearance of the toxic signs[5, 6] (FIGURE 2). This delayed reaction is the outstanding toxicological difference between streptomycin and streptothricin.

A similarly delayed onset of the toxic reaction is also observed in the topical application of streptothricin.[5] Instillation into a rabbit's eye of a buffered solution, containing 500–1,000 units per ccm. of either agent, is likely to cause an immediate inflammatory reaction. This is much more pronounced with streptothricin, but generally disappears with both drugs within the 12–24 hours following application. However, while, in the case of streptomycin, the eye remains normal thereafter,[4] a second and much more severe reaction appears, after an additional 24–48 hours, when streptothricin has been given.[5] It is characterized by an intense hyperemia and by the formation of a thin white membrane which completely covers the cornea. Even after 1–2 weeks,

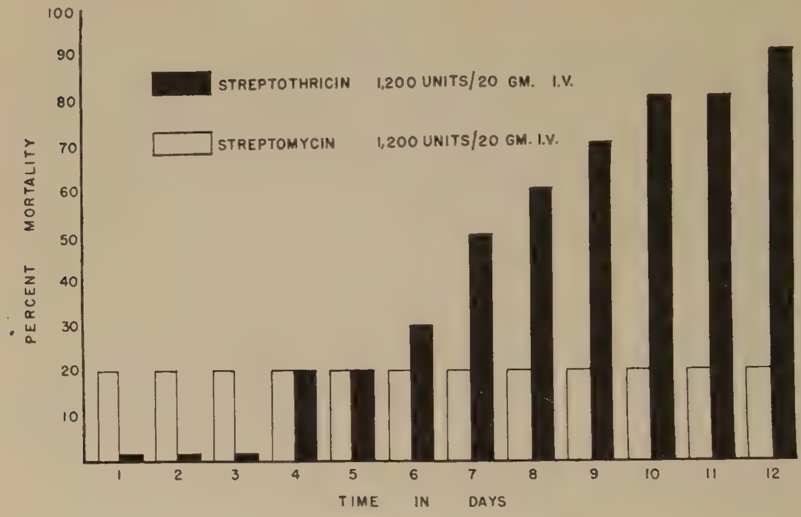

FIGURE 2. Comparison of delayed toxicity of streptomycin and streptothricin.

the palpebral fissure may still be shut by a mucoid mass of disintegrated white cells. Permanent loss of vision is the usual outcome of a topical application of a streptothricin solution of the above concentration. With streptomycin, a delayed reaction of this type has never been observed (PLATE 4).

The doses of streptothricin tolerated for several weeks by mice and rats are relatively small (below 5000 units per kgm. per day). Larger doses cause loss of body weight and muscle tone, gross hematuria, a steady decrease of urinary volume, and eventual death from renal insufficiency. Similar toxic effects are observed in dogs and monkeys, which, on a body weight basis, are even more sensitive than the smaller species.[5]

Streptomycin, on the other hand, is tolerated by mice and rats, in average daily doses up to 200,000 units per kgm., for periods up to several months.[4] Since the units for streptothricin and streptomycin are practically of the same order of magnitude, the much higher toxicity of streptothricin becomes immediately apparent.

When streptomycin doses exceeding the tolerated limits are given, characteristic pathological changes are regularly observed, particularly in the larger animal species.[7] The hepatic and renal function becomes increasingly impaired, as judged by function tests and biochemical criteria.[8] In addition to this, neurotoxic signs may develop after administration of sufficiently large doses, consisting, in rats, of a high degree of hyperexcitability, restless, rapid, spasmodic, and uncoordinated movements;[9] in dogs, of a striking change in gait and posture and, possibly, impairment of hearing.[4] Autopsy of animals overdosed with streptomycin reveals a fatty metamorphosis of the liver and, less frequently, the kidneys (PLATE 5). These changes, however, appear to be reversible, if the drug administration is stopped in time.[4, 7]

Autopsy findings in dogs and monkeys injected with streptothricin show hepatic and renal changes, predominantly in the tubules, of a similar nature, although of greater severity and often progressing to complete necrosis[5, 10] (PLATE 6). In addition to these changes, congestion and extensive hemorrhages, particularly around the capillaries of the mucosa, are found along most of the intestinal tract, as well as ulcerative lesions with marked suppuration and necrosis in the tongue, lips, and buccal mucosa. Local necrosis occurs at the site of intramuscular or subcutaneous injections.[10]

The impairment of renal function, regularly observed in animals poisoned with streptothricin, appears to be closely related to the total amount of drug given. Thus, a single injection of 80,000 units per kgm. produced an almost complete anuria, on the sixth day after injection, while a single dose of 20,000 units per kgm. was ineffective in this respect. If, however, the number of injections was increased to 20,000 units per kgm. daily, for 3 days, and 10,000 units per kgm. daily, for 10 days, results similar to those of a single injection of 80,000 units were obtained[5] (FIGURE 3). No such cumulative or delayed effects were ever observed with streptomycin.[4]

In contrast to the difference in delayed effect, the immediate action upon water diuresis is very similar with both drugs and depends largely upon the purity of the preparation. A single dose of either agent may cause a temporary inhibition, after which, in the case of streptomycin, the diuretic response soon becomes normal; while, with streptothricin, the course of recovery depends upon the quantity administered.[5]

The acute effects of streptothricin and streptomycin upon the circulatory and respiratory systems are quite similar. Small doses cause a transient increase in the depth and frequency of respiration, while large doses exert a depressing effect. Intravenous injection of a preparation of average purity causes peripheral vasodilatation and a sharp fall in arterial blood pressure.[4, 5] These effects vary greatly with the purity of the preparation, the rate of injection, and the concentration,

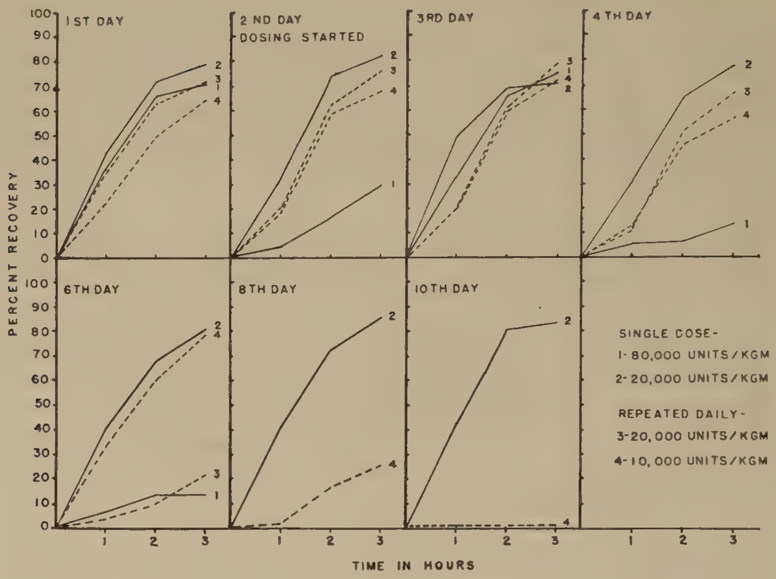

FIGURE 3. Effect of single and repeated doses of streptothricin on water diureses of rats.

being most pronounced when a concentrated solution is injected rapidly (PLATE 7A).

The principal pharmacodynamic effects of streptothricin and streptomycin concentrates of average purity may, thus, be summarized as follows: (1) Both cause acute death from asphyxia; (2) upon prolonged administration, both cause hepatic and renal damage; (3) both produce, upon parenteral administration, peripheral vasodilatation and a fall of blood pressure; (4) both temporarily inhibit water diuresis; (5) both produce local irritation, which, however, is much more severe with streptothricin.

The drugs differ decidedly, however, in their delayed effects. These are very marked and quite regular with streptothricin, while they are completely absent with streptomycin.

Streptomycin occasionally produces, upon repeated administration, neurotoxic disturbances, particularly of the auditory and vestibular systems.[4] Such disturbances have not been reported for streptothricin; however, re-examination of old protocols suggests that such changes may, actually, have been present and may have been ascribed to the debilitated condition of the animals and the presence of uremia.

The foregoing description provides a fairly complete account of the characteristic pharmacodynamic effects, which follow the administration of streptothricin or streptomycin of average purity. We shall now examine which of these effects are intrinsic properties of these antibiotics, and which may be ascribed to the "standard" impurities which accompany them.

There is ample experimental evidence that, at least, the acute intravenous toxicity, the circulatory effects, and the inhibition of water diuresis are largely influenced, if not entirely caused, by "standard" impurities. One of these is histamine-like, if not histamine itself. Its quantity varies considerably from lot to lot and depends, among other factors, upon the composition of the nutrient broth used for growing the parent molds, and the methods of extraction. This impurity may be destroyed by histaminase,[4, 8] an enzyme which inactivates histamine.

As may be seen from the difference in results obtained with the same preparation, before and after treatment with histaminase, this impurity is largely responsible for the circulatory effects (PLATE 7B) and the temporary inhibition of water diuresis (FIGURE 4). It has, however, no influence upon the acute intravenous and possibly subcutaneous toxicity, as shown by a comparison of histaminase-treated and untreated lots[4] (FIGURE 5). The fact that the smallest dose of histamine which regularly kills mice, upon intravenous injection, is at least 300 times larger than the amount of histamine found in the most

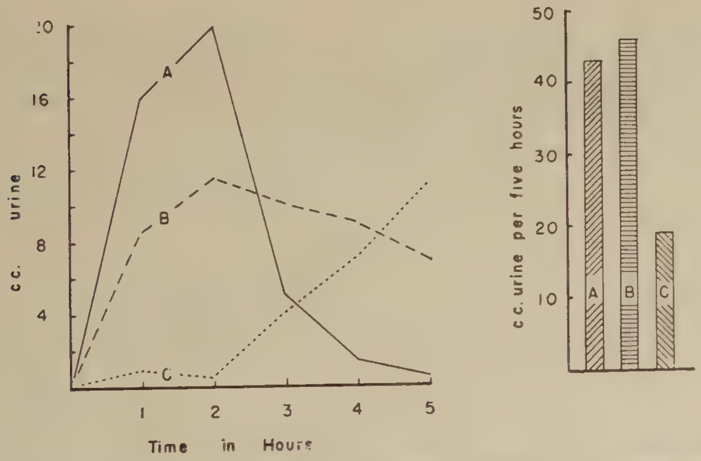

FIGURE 4. Effect of pure streptomycin and streptomycin concentrate on the water diureses of rats. Rate of excretion = curve on left. Total volume = columns on right.

A. Control 5 cc. water per 100 gm./kgm. s.c.
B. Pure streptomycin 250 mg./kgm. s.c.
C. Streptomycin concentrate 250 mg./kgm. s.c.

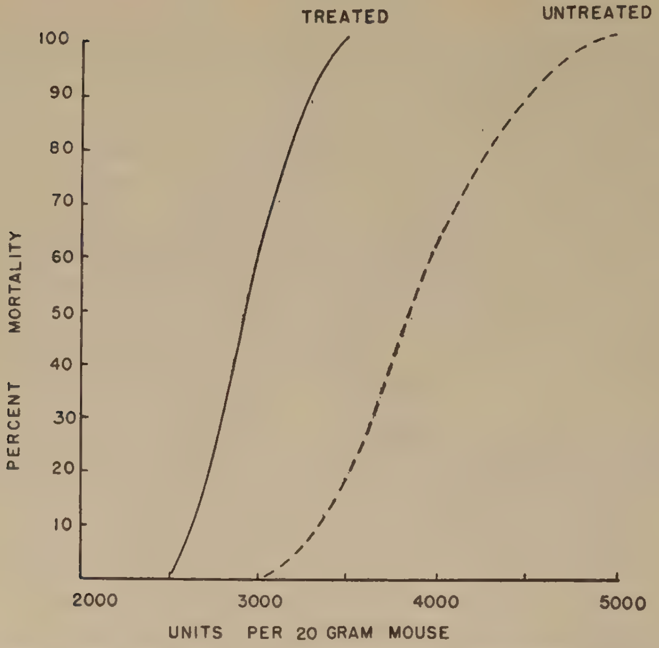

FIGURE 5. Intravenous toxicity of histaminase treated streptomycin concentrate.

heavily contaminated lot of streptomycin, serves as a further confirmation of this view.

The degree of contamination with the factor (or group of factors) responsible for the acute toxicity is largely independent of the potency of the preparation. Thus, we find both low and high potency lots with a high subcutaneous or intravenous toxicity. Indeed, even the most highly purified lots with potencies of approximately 750 units per mgm. vary considerably in their intravenous and subcutaneous toxicity.[4]

Since these highly purified lots contain only a very small amount of chemically unidentified substances and yet require only such small total doses as 2 mgm. per mouse, to produce acute death upon intravenous injection, it follows that we are dealing with an extremely toxic impurity. This may also explain the fact that the presence of this impurity cannot yet be detected by any chemical means, and that the only method for its qualitative and quantitative assay consists in an intravenous toxicity test. We have, at this time, no information regarding its chemical nature, nor do we know whether it consists of a single or of a group of contaminants. We can, however, postulate its existence as a separate and distinct entity, because, in contrast to the other impurity, it is not influenced by histaminase, and because even the most highly purified lots vary as much as 100%, in their intravenous and subcutaneous toxicity.

The toxic manifestations, following administration of streptomycin and streptothricin, may thus be divided into 3 groups: (1) Those due to the active principle itself; (2) those due to the histamine-like contaminant; (3) those caused by the highly toxic unidentified factor(s). The evaluation of the relative role of each of these elements in the composite, final, pharmacological reaction is complicated by the possibility that the specific action of each may be enhanced or decreased by the presence of the others. Thus, there is already experimental evidence that streptomycin is antagonistic to the histamine factor.[11]

The renal and hepatic changes following administration of streptothricin and streptomycin are apparently caused by the active principles of these agents, since they occur independently of all other pharmacodynamic effects and seem to be closely related to the total amount of drug administered. The delayed toxic reactions, characteristic of streptothricin, may also be regarded as a specific property of this antibiotic. Whether the neurotoxic signs, occasionally observed in animals[4] and man[12] after large doses of streptomycin, are caused by the agent itself (either due to overdosing or to an unusual sensitivity of the patient), or whether they must be attributed to an impurity, is not known, since insufficient amounts of highly purified lots have been available for investigation of this important question.

The pharmacological data just presented enable us to assess the relative safety of streptothricin and streptomycin for therapeutic purposes, and to evaluate the clinical significance of the two "standard" contaminants, the histamine-like factor and the factor responsible for the intravenous toxicity.

The pronounced and lasting pathological changes which are likely to result from the parenteral administration of even a single overdose of streptothricin would seem to constitute too great a risk to permit consideration of parenteral administration of this antibiotic. It may, on the other hand, be quite safe for topical use, including that upon the gastrointestinal mucosa after peroral administration, since it is not absorbed to any marked degree from the gastrointestinal tract.[5] Such topical use may be indicated, not only for sterilization of the gastrointestinal tract, but particularly for the treatment of dermal fungus infections which do not respond to streptomycin.[3]

The next question concerns the extent to which side-effects and complications may be attributable to the "standard" contaminants of streptothricin and streptomycin. Through close cooperation with clinical investigators, we have been able to study this problem by comparing the clinical reports, concerning individual lots, with the pharmacological results obtained with the same lots in animals. It soon became apparent that the one finding which permitted a reasonably accurate prediction of the clinical suitability of a particular lot, was its effect on the blood pressure. Lots which depressed it to any marked degree were frequently found to produce in patients nausea, vomiting, headache, flushing of the face and skin, and occasional fainting spells.[12-16] These undesirable, although generally not dangerous, side-effects were most likely to occur when highly concentrated solutions were injected directly into the blood stream.

There was much less agreement between clinical and animal findings, with regard to the intravenous toxicity and pyrogenicity. While lots producing in rabbits a typical pyrogenic reaction were obviously never sent to clinicians, there were, nevertheless, not infrequently reports of marked rises in body temperature after the first or second day of treatment, which disappeared after withdrawal of the drug.[13, 14, 15] No satisfactory explanation of this phenomenon can, as yet, be given, except that it seems to depend as much upon the sensitivity of the individual patient as upon the particular lot used. The same is true for the findings of severe arthralgia and skin rashes,[12, 14, 15] which, thus far, can not be correlated with findings in animal tests. Neither have we been able to evaluate the clinical significance of the impurity causing acute death upon intravenous injection, although there is, perhaps, a suggestion that neurotoxic disturbances may more frequently occur with lots of high intravenous toxicity.

The preliminary standards for streptomycin established by the Food and Drug Administration are based on the results of animal experiments and clinical findings. They exclude from clinical use lots which are excessively contaminated with the histamine factor, which have a high intravenous toxicity, and contain bacterial pyrogens (TABLE 1).

Considering the fact that all clinical work, to date, has been performed with samples of streptomycin containing varying amounts of at least two distinct contaminants, it seems justified to expect that the use of more highly purified preparations will still further reduce the undesirable side-effects, which, even now, are not frequent and have failed to create a serious obstacle in its use.

On the basis of its general pharmacological and toxicological properties and its extraordinary chemotherapeutic activity, the conclusion

TABLE 1

LETHAL TOXICITY OF HIGHLY PURIFIED STREPTOMYCIN SAMPLES

Sample number	*Potency*	*Blood Pressure*	*L.D. 50 Mgm./Kgm.*		Ratio of intravenous to subcutaneous L.D. 50
	Micrograms streptomycin base per mgm.	(Histamine-like) effect	Intravenous	Subcutaneous	
320	800	Negative	145	600	1:4+
8798	670	Negative	150	750	1:5
225	700	Negative	170	850	1:5
389	800	Negative	185	750	1:4
9803	750	Negative	250	1250	1:5
9767	750	Negative	300	1150	1:4−
LETHAL TOXICITY OF STREPTOMYCIN CONCENTRATES OF AVERAGE PURITY					
179	210	++	100	750	1:7.5
181	320	+	75	600	1:8
187	230	+	100	700	1:7
169	210	+++	100	400	1:4
174	280	++	125	1050	1:8+
184	260	+++	75	750	1:10
223	200	+	175	1250	1:7+

seems permissible that streptomycin is a safe chemotherapeutic agent. However, there still remains a great deal of experimental work to be done, before it can be used with a maximum of safety and efficacy. It must be kept in mind that, unlike penicillin, streptomycin as well as its impurities are pharmacodynamically very active compounds. A more detailed knowledge of their toxicologic properties is, therefore, a matter of great importance in the everyday use of the drug. This is in contrast to penicillin, where these aspects may be regarded as a question primarily of academic interest.

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14. **Heilman, D. H., F. R. Heilman, H. C. Hinshaw, D. R. Nichols, & W. F. Herrell**
1945. Am. J. Med. Sci. **210:** 576.
15. **Herrell, W. E., & D. R. Nichols**
1945. Proc. Staff Meet., Mayo Clin. **20:** 449.
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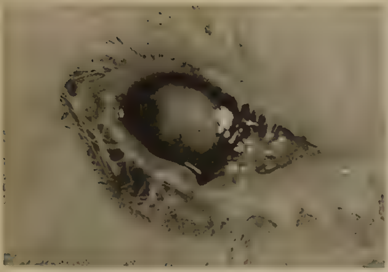
1945. J. A. M. A. **128:** 175.

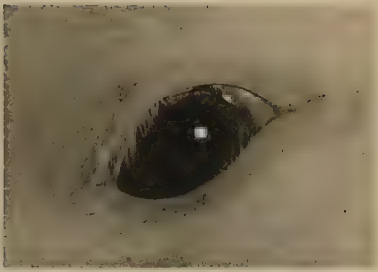
PLATES 4–7

Plate 4

Delayed effect of local application of streptothricin to rabbit eye.
Streptothricin (1000 units/cc.) instilled in left eye for 30 min. on 1/23/46.
Photographed 14 days later.



Left Eye
Streptothricin
Treated

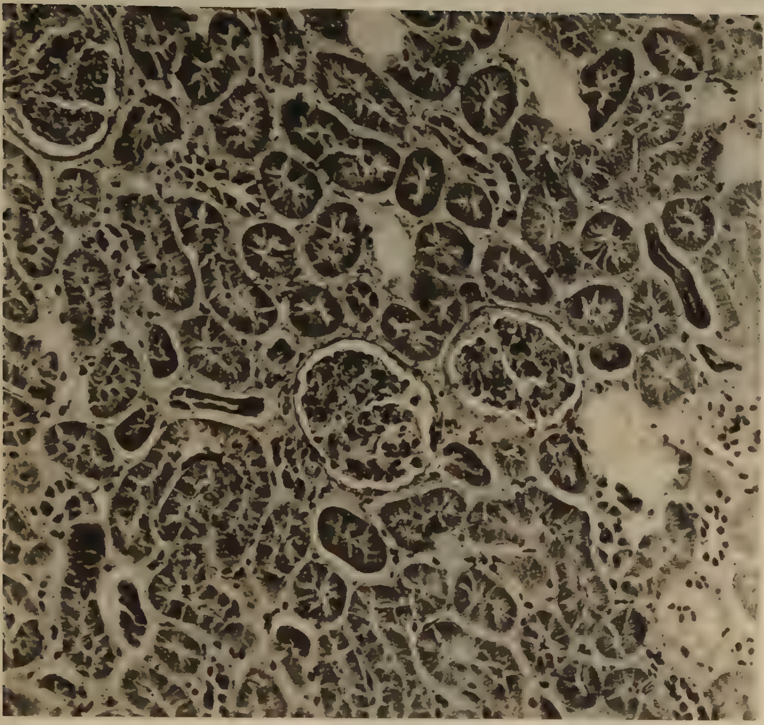


Right Eye
Control

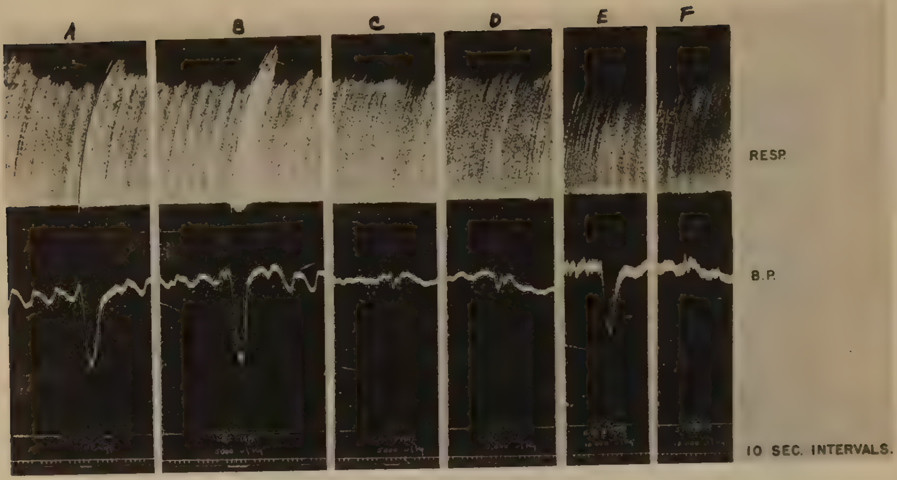
Plate 6

Kidney necrosis (streptothricin).

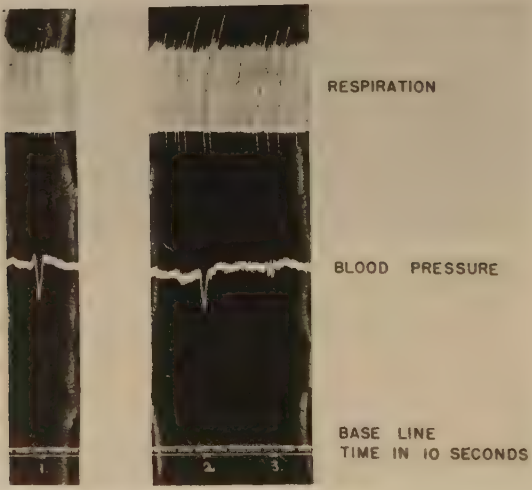
Kidney section of a monkey dosed orally with streptothricin (10,000 units/kgm./day), for 62 consecutive days. (H & E Stain.)



MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (1)



A



B

MOLITOR: STREPTOTHRICIN AND STREPTOMYCIN (1)

PLATE 7

A. Comparison of blood pressure effect of different lots of streptomycin.
Cat Wt. 3.43 kg.—Nembutal anesthesia, 30 mgm./kg. I. P.
(A) Histamine—0.01 mg.; (B) Streptomycin Lot 169—5000 U/kg.; (C) Streptomycin Lot 162—5000 U/kg.; (D) Streptomycin Lot 162—10,000 U/kg.; (E) Streptomycin 5R8798—10,000 U/kg.; (F) Streptomycin 5R8869—10,000 U/kg.

B. Circulatory effects before and after histaminase treatment.
Cat Wt. 3.25 kg.—Nembutal anesthesia, 35 mg./kg. S. C. 1. Histamine HCl—0.00005 mg./kg. 2. Streptomycin concentrate Lot 206—50 U/kg. 3. Streptomycin concentrate Lot 206, histaminase treated—50 U/kg.

STREPTOMYCIN AND STREPTOTHRICIN: THE ABSORPTION, EXCRETION, AND CHEMOTHERAPEUTIC PROPERTIES

By Harry J. Robinson

Merck Institute for Therapeutic Research, Rahway, N. J.

From the reports on the pharmacological and pathological effects produced in animals, following streptomycin and streptothricin administration, it is evident that the toxicity of these two substances differs in a number of respects.[1-4] In contrast, the antibacterial activity of streptomycin and streptothricin is remarkably similar, as shown by the fact that both antibiotics are mainly active against gram-negative bacteria *in vitro*[5, 6] and *in vivo*.[6-9] Moreover, both agents inhibit the growth of some gram-positive species, as well as certain acid-fast micro-organisms, including the human tubercle bacillus.[10, 11] However, the spectrum of antibacterial activity of streptomycin and streptothricin is not identical, as illustrated by the greater fungistatic activity of the latter,[8] also by the variation in the action of the two drugs against certain strains and species of bacteria.

The following discussion deals primarily with streptomycin, since this drug has been studied more extensively in the experimental animal, as well as in man. In addition, the behavior of the two drugs, with respect to absorption, excretion, and chemotherapeutic properties, appears to be almost identical. Therefore, in the interest of space, reference to streptothricin will only be made when the behavior of the two drugs is sufficiently different to warrant special comment.

Drug Administration

Aqueous solutions of each drug have been administered by a variety of methods, including continuous intravenous and intramuscular drip and intermittent intravenous and intramuscular injection, and by the subcutaneous, intrathecal, and peroral route. More recently, streptomycin has been given by nebulization. It will be noted that the studies on streptothricin have been confined to animals, whereas the results presented on streptomycin were obtained in both animal and man.

ABSORPTION

Although considerable information is now available on the absorption of streptomycin in animals[12] and man,[13-20] much less is known about this subject with regard to streptothricin.[2] All of the available information on both drugs has been obtained by using biological assay methods,[20-22] which have certain well-known limitations. In view of the latter, the findings reported in the present communication may subsequently be modified, either by improved biological assay procedures, or by the development of suitable chemical methods for the quantitative determination of these drugs in body fluids.

Single-Parenteral Injection

Observations of a number of investigators in animals have shown that, following the parenteral injection of a single dose of streptomycin, the drug concentration in the serum reaches a peak shortly after the injection, and thereafter decreases at a uniform, but relatively rapid, rate, over a 2 to 6 hour period. When large doses are given, the peak concentration is higher, and detectable amounts of streptomycin re-

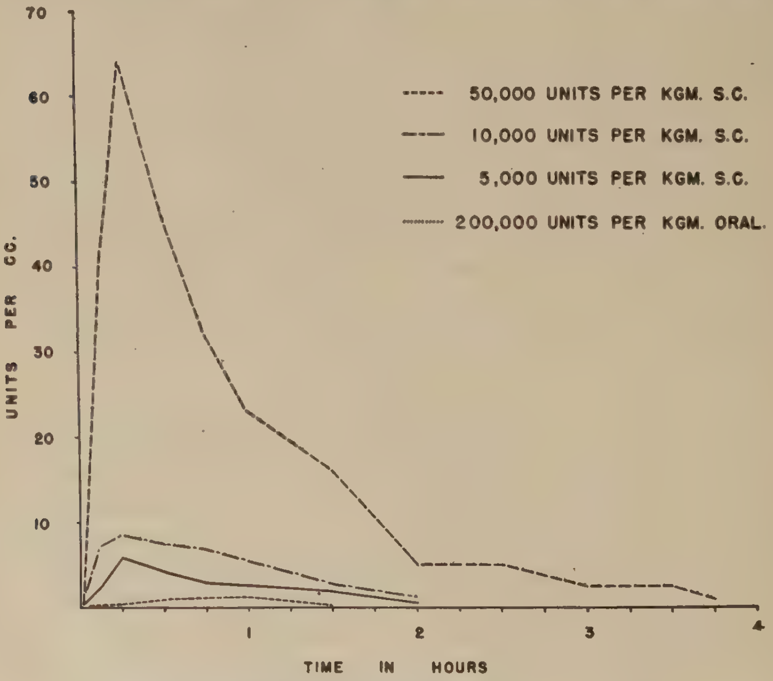


FIGURE 1. Blood concentration of streptomycin in mice.
(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. **60:** 68–72. 1945.)

main in the blood longer than when smaller doses are injected. For example, in mice, a single subcutaneous injection of 5 mg.* per kgm. produced an average blood concentration of 6.5 micrograms per ccm. within 15 minutes. Thereafter, the blood levels declined to approximately 2 micrograms per ccm., within a 2 hour period (FIGURE 1). Larger doses of 50 mg. per kgm. resulted in correspondingly higher and more prolonged blood concentrations, although, here again, the drug disappeared quite rapidly from the blood. In larger animal species, such as the dog and monkey, single intravenous injections of 100 mg. per animal (3.7 and 7.4 mg. per kgm.) produced average blood levels of streptomycin ranging between 20–30 micrograms per ccm., immediately following the injection. At the end of 4 hours, the blood concentration decreased to 2–3 micrograms per ccm. (FIGURE 2). Essentially the same results were obtained with streptothricin (FIGURE 3).

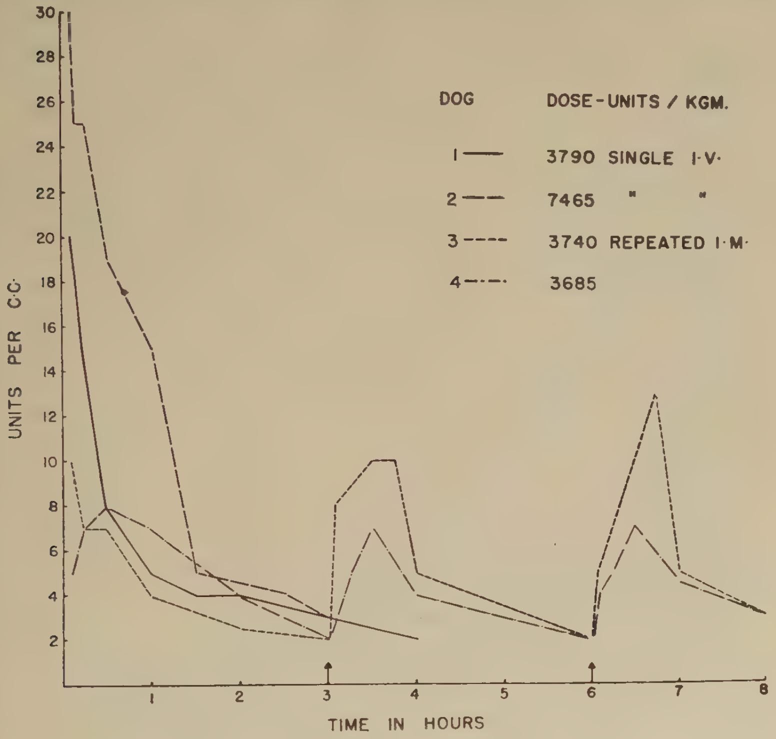


FIGURE 2. Streptomycin levels in the blood of the dog.
(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. **60:** 68–72. 1945.)

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* 1 microgram = 1 unit.

These observations have been extended to humans by a number of investigators.[13-20] In general, the absorption appears to be essentially the same as in the lower animal species. After intravenous injections of 600 mg. of streptomycin to patients, it was found that the average blood concentration was 32.8 micrograms per ccm., fifteen minutes after the injection. At the end of 6 hours, 4.9 micrograms per ml. were still found in the majority of patients.[14] When the same amount of drug was given as a single subcutaneous injection in 1% procaine solution, the maximum blood level was not attained until between 2 and 3 hours. Likewise, when administered by intramuscular injection, the maximum blood concentration was not as high as that obtained following the intravenous injection, but the blood concentration was maintained at a higher level over the 6 hour period.

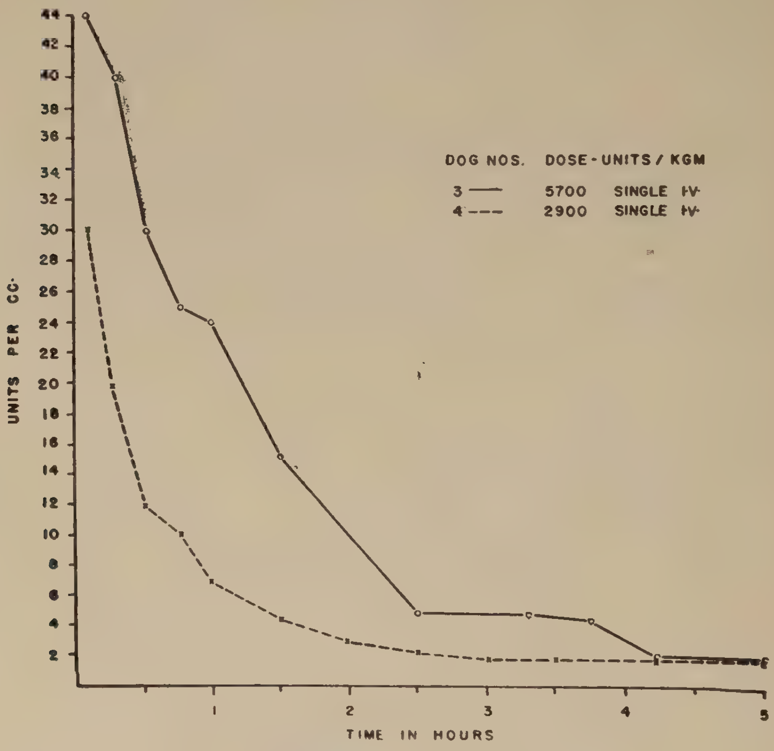

FIGURE 3. Streptothricin levels in the blood of the dog.

Smaller single doses of streptomycin produced correspondingly lower blood concentrations, which were not maintained beyond 3 hours.

Repeated Parenteral Administration

It is apparent, from the foregoing results, that therapeutic drug concentrations can be maintained in the blood for at least 3 hours, following injection of an adequate dose. By giving streptomycin or streptothricin every three hours by intramuscular injection, the blood concentration can be maintained at a therapeutic level, the latter depending upon the dose administered. This can be readily seen in monkeys and dogs, in which the intramuscular injection of 8 mg. every 4 hours afforded levels ranging between 5 and 18 micrograms (FIGURE 4).

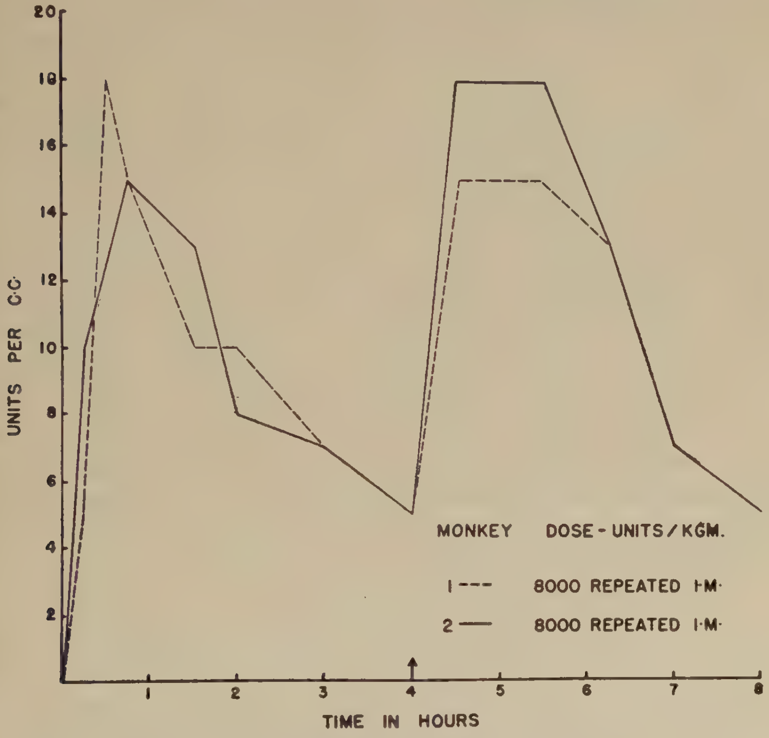

FIGURE 4. Streptomycin levels in the blood of the monkey.
(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.** Proc. Soc. Exp. Biol. & Med. **60:** 68–72. 1945.)

Zintel *et al.*[14] report that, in man, repeated administration of streptomycin every 3 hours leads to an additive or cumulative effect. Thus, the injection of 3.0 gm. per day in the form of divided doses resulted in blood concentrations between 20 and 60 micrograms per ml.

Hirshfeld[13] found some patients who developed a concentration of almost 200 micrograms per ccm. of serum, shortly after receiving doses of 1 gm. every six hours. As with other chemotherapeutic agents, therefore, it is advisable to follow the blood concentrations carefully.

Continuous Parenteral Administration

The methods of continuous intravenous and intramuscular infusion have been employed with considerable success. Of the two procedures, the intramuscular infusion may prove to be the method of choice, since the desired blood concentrations may be obtained without the occurrence of certain untoward reactions, which are occasionally seen following rapid intravenous administration. However, this may not be an important factor, when pure streptomycin becomes available for clinical use.

Although there have been no animal studies reported, to date, using the continuous drug infusion procedure, a number of reports have described the findings in man.[17] In most cases, the total daily dose of streptomycin was dissolved in physiological saline or 5% glucose solution, and given at a uniform rate over the 24 hour period. For example, some investigators[17] dissolved the total dose in 1000 ccm. of saline and regulated the flow to 8–10 drops per minute. Others used a greater volume of fluid. When 3.0 gm. of streptomycin, dissolved in 3000 ccm. of a 5% glucose solution, were administered daily by continuous intravenous infusion, Zintel *et al.*[14] obtained blood levels of streptomycin varying between 20 and 60 micrograms per ccm. of blood. Lower doses of 1.0 gm., by either the intravenous or intramuscular method, produced blood levels ranging between 10 and 20 micrograms of streptomycin per ccm. of blood. Slavin[20] reported somewhat higher values, under similar conditions.

Oral Administration

All reports agree that, when streptomycin is given by mouth to various animal species or to man, the drug concentration in the blood is low, even when relatively large doses are administered. In animals, similar results have been obtained with streptothricin. Heilman *et al.*[17] report that doses of 500 mg. of streptomycin per day by mouth did not produce detectable amounts of the drug in the blood serum of patients. Others[13] report that, in man, only 0.22 microgram of streptomycin was found in the blood, when 1.0 gm. was administered by this route. Studies with dogs show that relatively high blood levels can be obtained, in some animals, if doses of 30 to 50 mg. per kgm. are given by mouth. Smaller doses produce correspondingly lower levels.

Further evidence that some streptomycin or streptothricin is absorbed following oral administration, may be seen in experiments with infected mice. Thus, it is possible to protect animals infected with a

TABLE 1

TOTAL URINARY EXCRETION BY MONKEYS FOLLOWING DIVIDED INTRAMUSCULAR DOSAGE OF STREPTOMYCIN

(**Stebbins, R. B., O. E. Graessle, & H. J. Robinson.**[12] Proc. Soc. Exp. Biol. & Med. **60**: 68–72. 1945.)

Monkey No.	Time, days	Dose, units/kg × 1000	Total daily dose, units × 1000	Units excreted in 24 hours × 1000	% Daily dose excreted	Total % recovered
1	1	10	28.9	19.9	69	
	2	10	28.9	20.3	70	
	3	10	28.9	14.9	52	
	4	10	28.9	13.6	47	
	5	10	28.9	18.5	63	60
	6	—	—	4.		
	7	—	—	.4		
	8	—	—	1.		
	9	—	—	.6		
	10	—	—	.7		
	11	—	—	.0		65
2	1	10	32.4	19.8	61	
	2	10	32.4	20.5	63	
	3	10	32.4	17.1	53	
	4	10	32.4	16.4	51	
	5	10	32.4	24.5	76	60
	6	—	—	4.3		
	7	—	—	.8		
	8	—	—	1.1		
	9	—	—	1.2		
	10	—	—	.9		
	11	—	—	.0		66
3	1	50	167	70.	42	
	2	50	167	90.	54	
	3	50	167	78.4	47	
	4	50	167	72.	43	
	5	50	167	54.	32	44
	6	—	—	7.2		
	7	—	—	1.6		
	8	—	—	1.3		
	9	—	—	1.1		
	10	—	—	.6		
	11	—	—	.0		45
4	1	50	154	58.4	38	
	2	50	154	37.5	24	
	3	50	154	98.8	64	
	4	50	154	64.	42	
	5	50	154	32.5	21	38
	6	—	—	7.		
	7	—	—	1.4		
	8	—	—	.8		
	9	—	—	.6		
	10	—	—	.3		
	11	—	—	.0		39

streptomycin-sensitive pathogen such as *Salmonella schottmülleri*, provided large amounts of streptomycin or streptothricin are given by mouth.[6] It is evident, however, that the effective dose by mouth is approximately 30 times greater than the parenteral dose, and that these drugs are not readily absorbed from the gastrointestinal tract.

The low blood concentration of streptomycin following oral administration may be accounted for by an examination of the feces. Studies both in animals[12] and humans[19] indicate that much of the drug can be recovered in the feces, providing suitable extraction procedures are employed. Dogs given streptomycin perorally, on an empty stomach, in doses of 100 and 200 mg. per kgm., and sacrificed 24 hours later, showed that 60 to 80% of the drug remained unabsorbed from the gastrointestinal tract (TABLE 1). Some of the drug could be accounted for in the urine, but approximately 20–30% could not be recovered. It would seem reasonable to assume that a certain amount of streptomycin is inactivated in the animal, either by destruction or by the conversion into an inactive form. A second possibility is that the chemical extraction procedures employed and the assay methods are not satisfactory. Possibly, the availability of a chemical method of assay will aid in the elucidation of this problem.

Intrathecal Administration

For reasons which will become apparent later, in connection with the diffusion of streptomycin in body fluids, it is sometimes necessary to give streptomycin by the intrathecal route. By and large, the results of all investigators show that, following intrathecal administration, streptomycin remains in the spinal fluid for a long period of time. Thus, 24 hours after a single intrathecal injection of 15 to 20 mg., Buggs *et al.* report spinal fluid levels ranging from 13 to 25 micrograms per ccm. Similar findings were reported by others.[17] Clinical reports by Birmingham[24] and Heilman *et al.*[17] indicate that no untoward reactions appear, using this route of administration. On the other hand, Buggs *et al.*,[13] working with a single lot of streptomycin, report that all their patients treated intrathecally with streptomycin showed signs of meningeal irritation, as evidenced by the increased cell count and pain in the neck and head.

Administration by Nebulization

Preliminary experimental and clinical studies show that streptomycin can be given by nebulization, without producing irritation of the mucosa of the tracheobronchial tree. However, in spite of administering doses as large as 500 mg. daily, over a four week period, Heilman[17] and her co-workers could not find streptomycin in the blood serum, and the excretion of the drug in the urine was negligible. Considerable work remains to be done, before definite conclusions can be drawn regarding the usefulness of this route of administration.

Distribution of Streptomycin in Various Body Fluids

With few exceptions, streptomycin diffuses fairly rapidly into most body tissues, following parenteral administration.

Diffusion into Cerebrospinal Fluid

Diffusion of streptomycin into the cerebrospinal fluid does not take place readily. With doses of 1.0 gm. to 3.0 gm. per day, given by intermittent intramuscular injection, the cerebrospinal fluid levels ranged between 1–5 micrograms per ccm. The blood levels in these same patients ranged between 12–27 micrograms per ccm.[14] With smaller doses, the drug is not detectable in the cerebrospinal fluid. It is apparent, therefore, that, when high concentrations of streptomycin are desired in the cerebrospinal fluid, the drug must be given by the intrathecal route. The dosage and drug levels obtained by this method have been presented in a previous section of this paper.

Peritoneal Fluid

Following parenteral administration, streptomycin rapidly appears in the peritoneal fluid. Murphy and Ravdin[25] have shown that streptomycin enters the peritoneal cavity, both in normal animals and in animals with peritonitis. In man, most of the studies concerned with peritoneal fluid were performed on patients with ascites, due either to cirrhosis of the liver or to cardiac decompensation. In general, the results show that streptomycin first appears in the peritoneal fluid ½ hour after drug administration. Thereafter, the concentration increases, while the blood level decreases (FIGURE 5). Doses of 500 or 600 mg., given as a single intravenous or intramuscular injection, produce peak levels of 15 to 23 units per ccm. Smaller doses produce correspondingly lower fluid concentrations.

Pleural Fluid

In man, the appearance of streptomycin in pleural fluids appears to be somewhat slower than in the peritoneal fluid. Thus, fifteen minutes following a single intravenous injection of 600 mg., no streptomycin was found in the pleural fluid, despite a blood concentration of

30 micrograms per ccm.[14] Two hours later, there were only 6 micrograms of streptomycin per ccm. of pleural fluid, and 15 micrograms per ccm. in the blood. Buggs *et al.*[13] report that, after a single injection, streptomycin does get into the pleural fluid, but in rather low concentrations. Other studies show that, when streptomycin is given by repeated injection over a 24 hour period, higher pleural fluid concentrations are found, but there appears to be considerable variation from patient to patient.

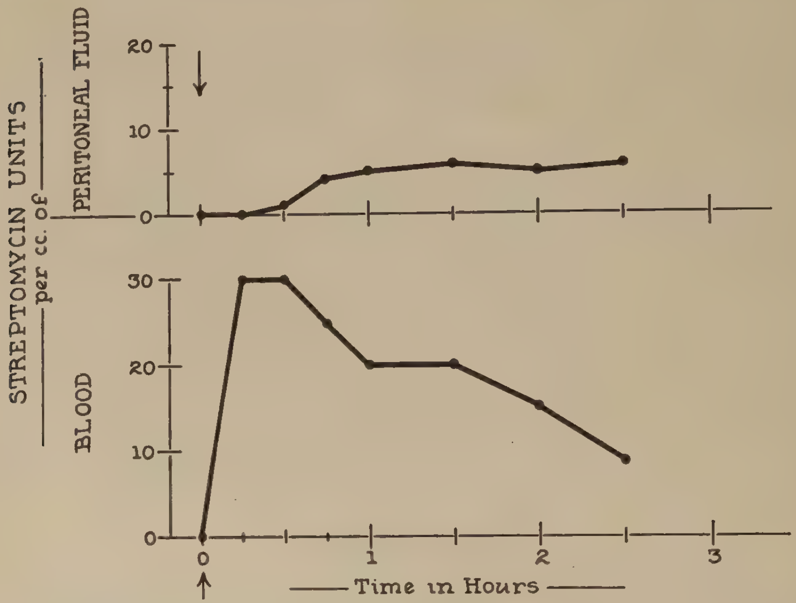

FIGURE 5. Diffusion of streptomycin into peritoneal fluid following a single intravenous injection of 600,000 units.
(**Zintel, H. A., H. F. Flippin, A. C. Nichols, M. M. Wiley, & J. E. Rhoads.** Am. J. Med. Sci. **210:** 421–430. 1945.)

Ocular Fluids

Studies in animals show streptomycin to be present in both the aqueous and vitreous humors of the eye, following intravenous and intramuscular injection or local application.[26] In a patient with glaucoma, 3 micrograms of streptomycin were found per ccm. of aqueous humor, 30 minutes following intravenous administration of 600 mg. The streptomycin level in the blood, at this time, was 40 micrograms per ccm.[14]

Fetal Blood and Amniotic Fluid

Both Heilman *et al.*[17] and Zintel *et al.*[14] agree that streptomycin does enter the fetal circulation and the amniotic fluid. Both groups of investigators administered streptomycin before the time of delivery and obtained specimens of fetal blood at the time of delivery. Zintel *et al.* found, in one patient, that the streptomycin concentration was 8 micrograms per ccm. of maternal blood; 9 micrograms per ccm. of cord blood; and 7 micrograms per ccm. of amniotic fluid, 2 hours after the intravenous injection of 250 mg.

EXCRETION

Renal Excretion

Following parenteral administration, streptomycin and streptothricin are readily eliminated from the body, the major portion being excreted in the urine. Experimental studies show that approximately 50–80% of streptothricin or streptomycin can be accounted for by renal excretion.[12] Similar findings have been reported for streptomycin, in man.[14–17] Streptomycin and streptothricin are rapidly eliminated from the body, provided fluid intake is adequate and renal function is normal. If renal function is impaired, as after the administration of streptothricin or, at times, with large doses of some lots of crude streptomycin, the drug accumulates in the body.

Quantitative studies on the rate of excretion of streptomycin and streptothricin show that most of the drug is excreted within the first 12 hours after the injection, but small amounts continue to be excreted as indicated above (FIGURE 6). Reimann *et al.*[19] report that the concentration which appears in the urine, at any given time, is inversely proportionate to the volume of urine. Hence, by restricting the fluid intake to 1200–1500 ccm. per day, it was possible to increase the streptomycin level in the urine of patients considerably. This procedure may be of importance for the treatment of urinary tract infection, where high urinary concentrates of streptomycin may be desirable.

Streptomycin and streptothricin have been isolated as active, colorless solids from the urine of dogs, by Tennent.[27] However, since, for the present, this material can only be characterized by pharmacological and bacteriological tests, it is not possible to state anything more about the urinary excretion products of streptomycin or streptothricin, beyond the fact that they possess the same antibacterial action as the parent substance.

Excretion of streptomycin also occurs in the bile, as shown by the fact that, in man, bile levels ranging between 7.5–12.5 micrograms per ccm. have been reported after the administration of from 100 to 125 mg. of streptomycin.[14, 17] Studies in animals show that 2–5% of the drug is excreted in the bile. Fecal excretion following parenteral administration is negligible, but accounts for most of the drug when oral administration is employed.

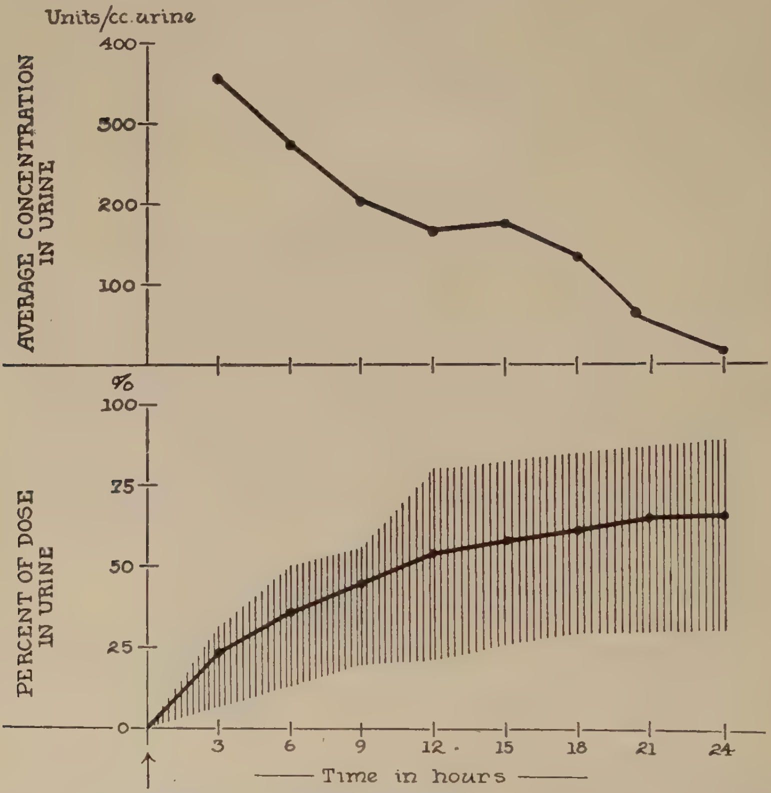

FIGURE 6. Urinary excretion of streptomycin following a single intravenous dose of 600,000 units; average of ten cases.
(**Zintel, H. A., H. F. Flippin, A. C. Nichols, M. M. Wiley, & J. E. Rhoads.** Am. J. Med. Sci. **210:** 421–430. 1945.)

DISTRIBUTION IN TISSUES

In view of the absence of a specific (chemical) quantitative test for streptomycin, this phase of the study is at present incomplete. Adcock[15] assayed various tissues of two patients who died of tuberculous meningitis while receiving streptomycin. His findings are presented in TABLE 2 and show most of the drug to be present mainly in the kidney or gall bladder bile.

TABLE 2

TISSUE LEVELS OF STREPTOMYCIN
(**Adcock, J. D.**, University of Michigan, Ann Arbor, Michigan.)

Tissue	Micrograms of streptomycin per gm.	
	Case I	Case II
Post-mortem serum	12	43
Gall bladder bile	21	—
Kidney	20	95
Lung	6	6
Heart muscle	1	5
Brain	0	trace
Liver	0	trace

CHEMOTHERAPEUTIC ACTION

In vitro Studies

An abundant literature has accumulated, in the few years since the discovery of streptothricin and streptomycin, concerning the action of these agents *in vitro* against a variety of bacteria, fungi, viruses, and protozoa. A good deal of the available data is conflicting and requires further interpretation, because of the different bacterial strains and techniques used by the various investigators. Despite these difficulties, certain definite conclusions may be drawn. Streptomycin and streptothricin in small quantities are bacteriostatic for a variety of gram-negative and gram-positive bacteria (TABLE 3). In addition, the tubercle bacillus is sensitive to both substances.[10, 11, 28, 29, 30] It will be noted that certain species of a given genus are more resistant than others, and, at times, this spread of resistance may be considerable. Other bacterial species, including the pathogenic spore-bearing anaerobes,[6] as well as the fungi,[6, 31] viruses,[32, 33] and certain protozoa[34–37] are also resistant to streptomycin and streptothricin. On the other hand, certain fungi are inhibited by streptothricin in concentrations ranging between 250 and 500 micrograms per ccm.[6] These results are not modified by the presence of blood or other body fluids.

Action *in vivo*

In general, organisms which are sensitive to streptomycin or streptothricin *in vitro,* are also sensitive *in vivo.* Some of the experimental

Table 3

Bacteriostatic and Fungistatic Activity of Streptomycin Agar-Streak Test

Gram-negative organisms	No. of strains tested	γ of streptomycin per ml. required for inhibition
E. coli	5	3.75–7.5
E. typhosa	9	7.5 –120
B. acidilactici	1	60
S. aertryche	1	30
S. enteritidis	2	3.75– 30
S. schottmülleri	2	15– 30
B. flexneri D	1	1.875
B. sonne	1	7.5
P. leptisepticum	1	3.75
B. proteus	4	30
B. pyocyaneus	5	60–>400
A. aerogenes	3	3.75– 60
H. pertussis	1	>240
H. influenzae	3	3.75
B. friedländeri (*Klebsiella pneumoniae*)	6	7.5–>240
S. typhi-murium	1	15
K. rhinoscleromatis	1	0.9
S. panama	1	30
B. viscosum	2	3.75– 15
Alk. fecalis	1	60
Paratyphoid A	1	30
Paratyphoid B	1	120
V. cholera	1	15
V. metchnikoni	1	7.5
Sarcina lutea	1	7.5
N. meningitidis Type I	1	1.875
N. meningitidis Type II	1	1.875
N. meningitidis Type IIα	1	7.5
Xanthomonas juglandis	1	1.875

infections in which streptomycin or streptothricin have been tried are those associated with the following organisms or diseases: The colon-typhoid group,[6, 9] *Salmonella* infections,[6, 9] brucellosis,[38, 7] tularemia,[39, 40] tuberculosis,[41, 42] *Klebsiella*,[43] *Proteus*,[44] and *Pseudomonas pyocyanea* infections; rabbit syphilis,[45, 46] leptospirosis,[47] and experimental relapsing fever;[47] murine pertussis[48] and streptococcal staphylococcal, and pneumococcal infections.[6] In addition, the drugs have also been tried in certain virus, fungus, and protozoan diseases, without success.

Experimental Colon-typhoid and Salmonella Infections in Mice

Among the bacterial infections which respond to streptomycin and streptothricin therapy, are those produced by most strains of *Escherichia coli, Salmonella schottmülleri, Salmonella aertrycke, Eberthella typhi* and *Aerobacter aerogenes*.[6, 7] In mice, single doses of 2.5 to 5.0 mg.

TABLE 3 (Continued)

Gram-positive organisms	No. of strains tested	γ of streptomycin per ml. required for inhibition
Corynebacterium pseudodiphtheriae	1	3.75
Corynebacterium diphtheriae (avirulent)	2	1.87–3.75
Corynebacterium xerosis	2	3.75
Corynebacterium hoffmanni	1	7.5
Corynebacterium diphtheriae gravis	1	15
Corynebacterium ovis	2	3.75
Corynebacterium pseudotuberculosis	1	15
Corynebacterium renalis	1	7.5
Corynebacterium pyogenes	2	7.5
Corynebacterium equi	3	3.75
Strep. fecalis	1	60
Strep. equi	2	15
Strep. hemolyticus	4	15–>120
Strep. viridans	2	120–>120
Strep. lactis	1	30
Strep. liquefaciens	1	240
Staph. aureus	7	3.75–>120
D. pneumoniae I	1	30
D. pneumoniae II	1	30
B. mycoides	1	3.75
B. subtilis	1	1.875
Anaerobes		
Cl. welchii	1	>104
Cl. tetani	1	>104
Cl. septicum	1	>105
Cl. sordelli	1	>105
Pathogenic Fungi		
Coccidioides immunitis	1	>8000
Monilia albicans	1	>8000
Cryptococcus neoformans	1	>4000
Epidermophyton inguinale	1	>4000
Microsporum canis	1	4000
Sporotrichum schenchii	1	>4000
Trichophyton gypseum	1	3500
Trichophyton mentagrophytes	3	4000

per kgm. of either drug, given intravenously or subcutaneously immediately after the bacterial inoculation, were sufficient to protect a large percentage of the mice from a lethal infection of the foregoing organisms. Under similar conditions of infection, the drugs were even more active when given by intraperitoneal injection, but considerably less active when given by mouth.[6] Essentially the same results were obtained when streptomycin or streptothricin was given in the form of multiple doses every 6 hours (TABLE 4).

TABLE 4

EFFICACY OF STREPTOMYCIN IN MICE INFECTED WITH *Salmonella schottmülleri* BY SUBCUTANEOUS THERAPY

(**Robinson, H. J., D. G. Smith, & O. E. Graessle.**[6] Proc. Soc. Exp. Biol. & Med. **57**: 226–231. 1944.)

Organism, *Salmonella schottmülleri;* Age of Culture, 6 hours; Infection, 0.5 c.c. of a 10^{-5} dilution in 4% mucin; Therapy, Streptomycin or streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days 1	2	3	4	5	6	7	8	Survival %
				Therapy: Single dose									
10	Streptomycin	12.5	1	10^{-5}	2	2	2	2	2	1	1	1	10
10		25.0	1	10^{-5}	8	8	8	7	7	7	7	7	70
10		50.0	1	10^{-5}	10	10	10	9	9	9	9	9	90
10		100.0	1	10^{-5}	9	9	9	9	9	9	9	9	90
10	Streptothricin	12.5	1	10^{-5}	4	3	2	2	2	2	2	2	20
10		25.0	1	10^{-5}	5	4	4	4	4	4	4	4	40
10		50.0	1	10^{-5}	10	10	10	9	9	9	9	9	90
10		100.0	1	10^{-5}	10	10	10	10	10	10	10	10	100
				Therapy: Every 6 hrs. over a 24 hr. period									
20	Streptomycin	12.5	4	10^{-5}	15	12	12	12	12	12	11	11	55
20		25.0	4	10^{-5}	20	19	18	18	18	18	18	18	90
20		50.0	4	10^{-5}	20	20	20	20	20	20	20	20	100
10	Streptothricin	12.5	4	10^{-5}	9	5	4	4	4	3	3	3	30
10		25.0	4	10^{-5}	10	10	10	10	9	9	9	9	90
10		50.0	4	10^{-5}	10	10	10	10	10	10	9	9	90
10		100.0	4	10^{-5}	10	10	10	10	10	10	10	10	100
				Therapy: None									
20	Controls	—	—	10^{-5}	0	0	0	0	0	0	0	0	0
15		—	—	10^{-6}	6	4	3	3	3	3	3	3	20
15		—	—	10^{-7}	10	8	7	6	6	6	6	5	33
15		—	—	10^{-8}	12	10	10	10	10	9	9	9	60

Experimental Brucellosis

Studies in the infected chick embryo by Jones,[7] and in the guinea pig by Live *et al.*,[38] suggest that streptomycin is an effective agent for the treatment of experimental infections due to *Brucella abortus.* Live and his co-workers[38] report that the treatment of infected guinea pigs with 5.0 mg. of streptomycin, in the form of divided doses, had a definite bacteriostatic effect upon *Brucella abortus.* Larger doses of 30 mg. appeared to eliminate the infection entirely, in most animals, as determined by autopsy and culture procedures. It is important to recognize, however, that the lesions produced in man and the experimental animal are not identical and that, therefore, it is not possible to predict what influence streptomycin will have on brucellosis in man.

Experimental Tularemia

According to Foshay,[49] *Pasteurella tularensis* is extremely sensitive to streptomycin. *In vitro* studies show that as little as 0.25–0.4 microgram per ccm. is sufficient to inhibit the growth of this organism, whereas 2.0 micrograms are bactericidal. Studies by Heilman *et al.*[39] and Chapman *et al.*[40] indicate that streptomycin is of unquestionable value in controlling experimental tularemia in animals. The minimal effective dose given every 3 hours over an 8–10 day period to mice, rats, and monkeys was found to be 10, 10, and 2.5 mg. per kgm. respectively.[40]

Experimental Tuberculosis

The findings in tuberculosis will be discussed by Drs. Hinshaw and Feldman. Therefore, I will not consider the results obtained with streptomycin in this disease. I believe that, while the results may appear encouraging, a final decision as to the probable value of this drug in tuberculosis cannot be made at the present time.

Experimental Infections Caused by *Pseudomonas pyocyanea* and *Proteus*

The efficacy of streptomycin or streptothricin against these organisms varies considerably with different strains. In general, however, both organisms are quite resistant to streptomycin and streptothricin and, in addition, have marked potentialities for acquiring resistance to these drugs. In our experience, it was not possible to protect mice infected with a lethal dose of either organism, even when maximal amounts of the drugs were administered. However, in urinary tract infections due to *Proteus,* it is possible to obtain good results, due to the high concentration of streptomycin in the urine. following parenteral administration.[12]

Experimental Infections Due to Gram-Positive Bacteria

Cocci. Animals infected with *Staphylococcus aureus, Streptococcus hemolyticus,* and *Diplococcus pneumoniae* can be protected with large amounts of streptomycin. Studies in mice show that from 10 to 30 times as much streptomycin is required to protect against a lethal dose of the foregoing organisms as required for most gram-negative bacillary infections. Similar results have been obtained with streptothricin, but, with this drug, the effective dose was in the toxic range.

Anaerobes. The group of anaerobic sporulating bacilli, including *Clostridium tetani, Cl. welchii, Cl. sordelli,* and *Cl. septique,* is quite resistant to the action of streptomycin and streptothricin. *In vivo* studies in experimental *Clostridium welchii* infections in guinea pigs show streptomycin to have no influence on this disease, even when the drug was injected at the site of the infection.

Diphtheria. Although *Corynebacterium diphtheriae* and related species are quite sensitive to streptomycin *in vitro,* little is known about the influence of the drug on the organism *in vivo.* Much of the difficulty in the evaluation of drugs in experimental diphtheria lies in the fact that the disease in animals is not a localized infection in the nasopharynx, but, rather, a diffused generalized toxemia, resulting from large amounts of diphtheria toxin.

Spirochetal Infections. The available data on the effect of streptomycin and streptothricin against *Treponema pallidum,*[45, 46] experimental relapsing fever, experimental Weil's disease, and rat bite fever are not very abundant, at the present. The preliminary data suggest, however, that streptomycin has some effect on spirochetal infections, but this effect is considerably inferior to that of penicillin.

Fungi. In vitro tests show that streptothricin is much more active than streptomycin as a fungicide (TABLE 5). Concentrations of streptothricin between 250–500 micrograms per ccm. of agar completely inhibit a variety of pathogenic and saprophytic fungi, whereas, under the same conditions, streptomycin has no inhibitory effect, even in concentrations 10–20 times that of streptothricin. Preliminary *in vivo* tests suggest that streptothricin does not influence the course of experimental *Sporotrichum schenckii* infections in rats.[52] Likewise, streptomycin has been reported to have no effect on *Histoplasma capsulatum.*[31]

Protozoa. Streptothricin and streptomycin were not found active in experimental malaria,[35] rat filariasis,[53] or trypanosomiasis.[6, 8]

TABLE 5

BACTERIOSTATIC ACTION OF STREPTOMYCIN AND STREPTOTHRICIN AGAINST FUNGI*

Agar Plate Method: Sabouraud's Agar. (**Robinson, H. J., D. G. Smith, & O. E. Graessle.**[6] Proc. Soc. Exp. Biol. & Med. **57**: 226–231. 1944.)

Organism	No. of units per ccm. agar required to cause inhibition	
	Streptomycin	Streptothricin
Aspergillus niger MF II	>4,000	250
Penicillium chrysogenum MF 56	>4,000	250
Cryptococcus neoformans No. 3709	>4,000	250
Epidermophyton inguinale	>4,000	1,000
Microsporum canis No. 232	4,000	1,000
Sporotrichum schenkii No. 7017	>4,000	250
Trichophyton gypseum	3,500	500
Trichophyton interdigitale No. 640	4,000	1,000

* Cultures were incubated at 29° C. for 14 days.

Viruses. Neither agent has any influence on PR8 strain of epidemic influenza.[32, 33]

Oral Administration

The results obtained in our study demonstrate the marked activity of streptothricin and especially streptomycin in the reduction of the number of organisms in the intestinal tract of mice.[50, 51] Not only were the numbers of the coliform organisms rapidly and effectively reduced, but also, in the case of streptomycin, the total flora was strikingly decreased (FIGURE 7). The results of this study suggest that streptomycin might be effective, not only as a chemotherapeutic agent for enteric diseases, but also as a form of preventive medicine in the preoperative and postoperative treatment of surgical infections of the gastrointestinal tract.

ACQUIRED RESISTANCE TO STREPTOMYCIN

In concluding this discussion, it seems worthwhile to mention briefly the importance of the phenomena of fastness in its relationship to streptomycin therapy. A number of workers have been able to produce streptomycin fastness in organisms previously susceptible, by culturing them in a medium in which the concentration of streptomycin was progressively increased. Graessle and his associates were able to

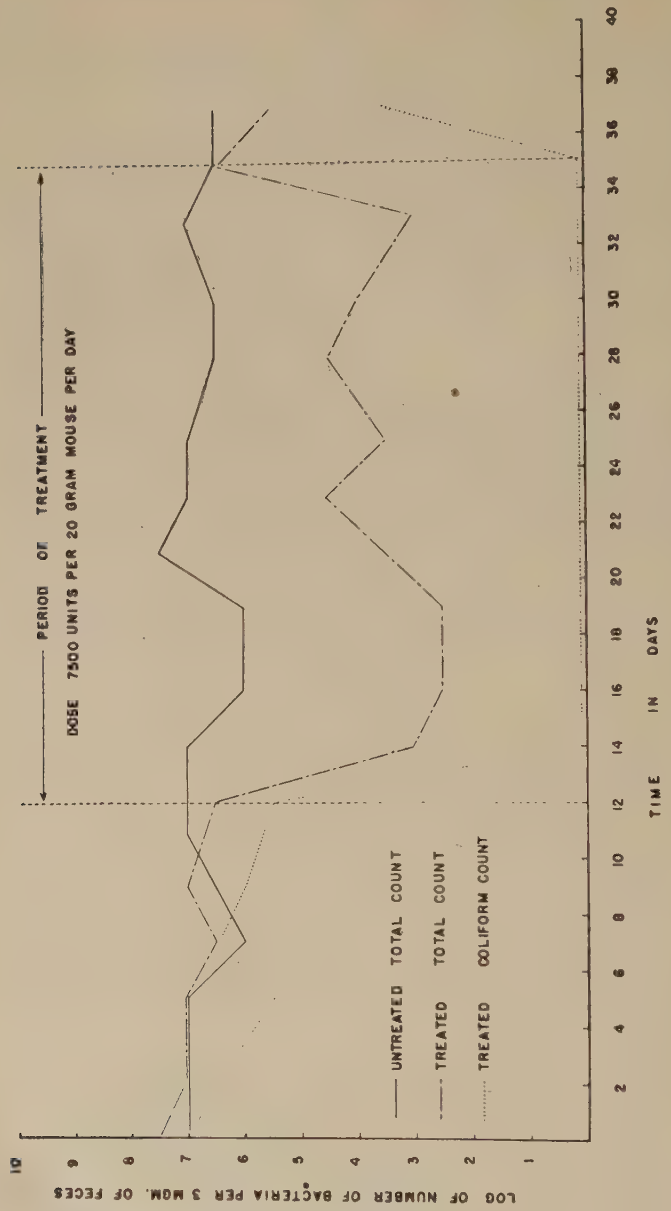

FIGURE 7. Influence of streptomycin on the bacterial flora of mice. (**Smith, D. G., & H. J. Robinson.** J. Bact. **50:** 6. 1945.)

increase streptomycin resistance in strains of *Escherichia coli, Salmonella schottmülleri,* or *Staphylococcus aureus* several thousand-fold, by subculturing the organism in this manner for a period of several weeks. These investigators showed that the degree and rate at which five individual strains of *Staphylococcus aureus* developed resistance to streptomycin were more uniform and rapid than that which occurred with penicillin. Moreover, it was possible to induce resistance in a strain of *Salmonella schottmülleri* without alterations in the animal pathogenicity or virulence. It is of interest to note that, when resistant strains of *Escherichia coli* were subcultured in a medium free of streptomycin, the organisms began to return to their original sensitivity (FIGURE 8). This is contrary to the findings of Demerec,[54] who worked with a single culture of *Staphylococcus aureus* and found the acquired resistance to remain through 20 transfers.

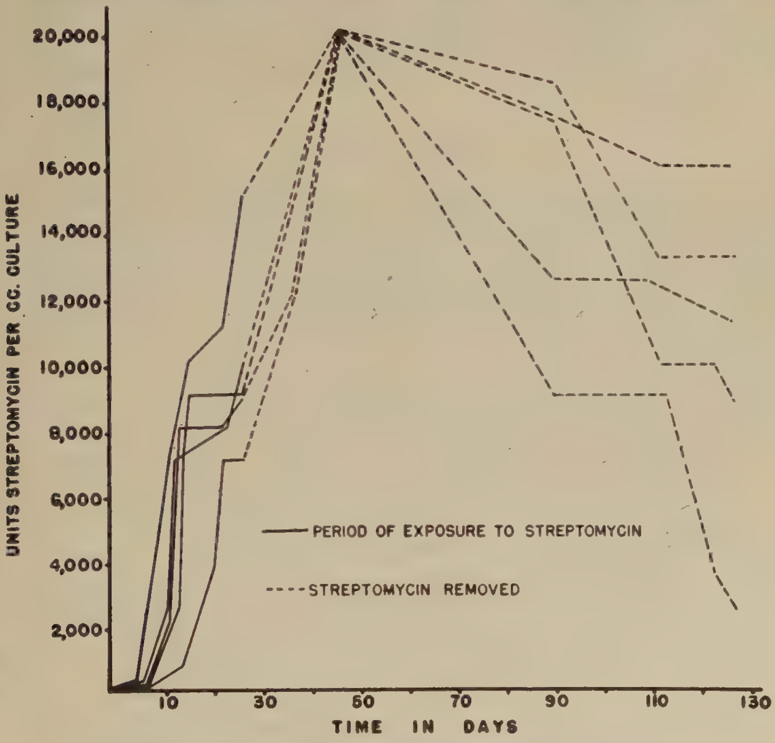

FIGURE 8. Acquired resistance of five strains of *E. coli*-W to streptomycin *in vitro.* **(Graessle, O. E., & B. Frost.** To be published.)

Although much work appears to be in progress on the subject of fastness, little has been reported on the mechanisms involved in this phenomenon. Demerec postulates two possible mechanisms for the development of penicillin-resistant organisms, which might apply equally well to streptomycin: (1) Resistance is an acquired characteristic which develops through the interaction between bacteria and penicillin, when the two are in contact with each other; and (2) resistance is an inherited characteristic which originates through mutation, and its origin is independent of penicillin treatment. In either case, it is possible, with most organisms, to prevent the occurrence of fastness by exposing the organism to adequate bacteriostatic or bactericidal concentrations of the drug. In the light of this, it is of paramount importance to institute adequate therapy in all patients, if the phenomena of acquired resistance are to be prevented.

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PHARMACOLOGY OF PENICILLIN

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I. GENERAL

As originally used by Fleming,[1] the term, *"penicillin,"* described the total antibacterial activity produced by a particular mold. It is now known that a number of unrelated chemical substances may be produced by the same organism, and the term has come to mean a group of chemical compounds, to which the chief antibacterial activity is due, having one or other of the general formulae shown in FIGURE 1. At least six types of penicillin are now recognized (FIGURE 2), differing from each other only in variations of R.[2–5]

The type of penicillin present in commercial samples varies greatly Under different conditions, one or another of the above types can be produced predominantly. At the present time, it is impossible to give more than an approximate idea as to what the relative composition of any given sample may be. When penicillin was being produced in surface cultures, type F predominated. Later, when deep fermentation first came into use, G was the predominant type. Following changes in strain of mold and in methods of fermentation and extraction, commercial preparations now ordinarily contain a mixture which is mostly F, K, and G penicillins. Penicillin X is usually present in only very small amounts, except in "penicillin" produced by surface culture.

Much of the original work by the Oxford group was carried out with extremely impure material, assaying in the neighborhood of 50 units per milligram, or less. Subsequently, there has been steady improvement in the purity of penicillin. Thus, from records taken by a single producer, between July 1942–June 1943, the potency of 42 batches was from 69 to 333 units per milligram. At the end of 1945, 100 consecutive batches, from the same producer, averaged 1087 units per milligram, ranging from 900 to 1200 units per milligram.[6] Coghill and Koch,[7] also, note a similar trend for the industry, as a whole. In addition, the minimum requirements of the Food and Drug Administration for penicillin potency have progressively been raised, and, on September 1, 1945,[8] were raised from 300 to 500 units per milligram.

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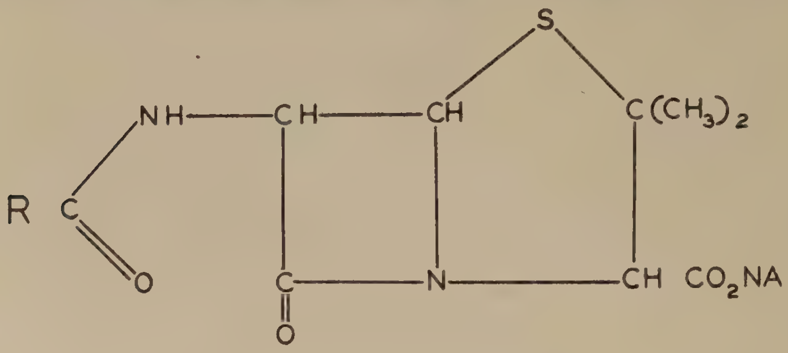
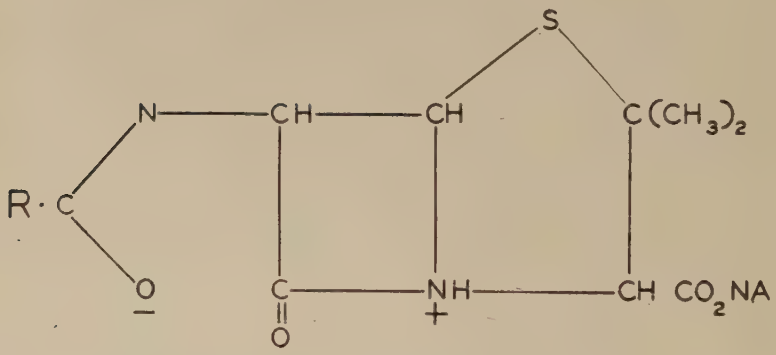


I

β LACTAM STRUCTURE



II

INCIPIENT AZLACTONE STRUCTURE

FIGURE 1.

In spite of this, commercial penicillin today must still be considered a highly impure substance, since the very best preparations still contain some 25% of material which is, chemically and biologically, not active penicillin.

Chemical Properties

The various, pure, free penicillins are strong acids, having pK's in the neighborhood of 2.8. They readily form salts with the ordinary cations, of which sodium and calcium are most commonly prepared. The latter has a special interest, since it is the least hygroscopic and most stable of the commonly available salts, and is particularly well suited for the manufacture of tablets and oil-beeswax mixtures. Penicillin, in the form of the free acids or salts, is highly soluble in water, and most salts are hygroscopic. The free acids are also soluble in organic solvents and fats, this property serving as an important means by which penicillin can be separated from crude broth or biological fluids.

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[8] Federal Register. **10:** 11231. 1945.

TYPES OF NATURAL PENICILLINS

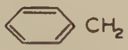
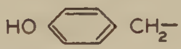
NAME	SYNONYM	SOURCE	R-
F	I	P CHRYSOGENUM-NOTATUM	$CH_3CH_2CH{=}CHCH_2-$
DIHYDRO F	GIGANTIC ACID	A GIGANTEUS	$CH_3CH_2CH_2CH_2CH_2-$
FLAVACIDIN	F TYPE	A FLAVUS	C_5-
G	II	P CHRYSOGENUM-NOTATUM	 CH_2
X	III	" "	 HO CH_2-
K	IV	" "	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2-$

FIGURE 2.

It is possible to esterify the free carboxyl group, without destroying the basic structure of the molecule, and the methyl, ethyl, butyl, benzyl, and benzhydryl[9, 10] esters have been prepared. Such compounds are of interest, because they are almost completely insoluble in water, but possess high fat solubility. They have little, if any, antibiotic activity, until they are hydrolyzed.

Stability

One of the chief obstacles to the production of penicillin is its instability, particularly when it is present as the free acid. It is also readily destroyed at pH's of 8.5 or higher. The basic structure is destroyed by alcohols, particularly methanol, by sulfhydryl compounds, by heat,

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and by repeated freezing and thawing. In addition, many bacterial species produce an enzyme, penicillinase,[11] which has the capacity of destroying the basic molecule. At one time, there was evidence that, when the strain, method of fermentation, and method of extraction were kept constant, stability of the product increased with increasing purity. Unfortunately, when any of the above conditions was modified, any correlation between potency and stability was lost.[12]

Different Penicillins

The chemically different penicillins undoubtedly differ, also, in their biological characteristics. Unfortunately, however, because of lack of sufficient amounts of material, this point has been little studied. A study has been made of the inhibitory activity of pure preparations against certain bacteria. TABLE 1 shows the activity of pure crystalline preparations of the various penicillins per mg., in terms of international standard units,[13] *i.e.,* measured against one or the other of two specifically described strains of staphylococcus, against *Staphylococcus aureus* and, also, against a particular rough variant of a certain strain of *Bacillus subtilis*.[14] Finally, the ratio of the activity against *B. subtilis* (rough) to the activity against the standard strain of staphylococcus, is given. From this, it is seen, for example, that X is nearly twice as active against the former as against the latter, while K has only a third of the activity.

TABLE 1

Penicillin type	Activity in units/mg. against:		Ratio of activity
	Staph. aureus	*B. subtilis* (R)	v. *B. subtilis*/ v. *Staph. aureus*
G(II)	1667*	1667*	1.0*
F(I)	1465[14]	970[14]	0.65[14]
Flavacidin	1400[15]	1000[15]	0.72[15]
X(III)	850[7]	1450[14]	1.4 to 2.0[14]
K(IV)	2300[7]	760	0.33[7]

* By definition.

This higher ratio of activity for X, as against other penicillins, has held when organisms, other than *Bacillus subtilis* (R), are compared with *Staphylococcus aureus*. Thus, X was found more effective *in vitro,* on the basis of staphylococcal international units, but not necessarily on the basis of weight (X having approximately only half the number of such units per mg.), against *Klebsiella pneumoniae* and *Bacillus cereus,*[16] than was "commercial penicillin." However, since the composition of this penicillin was unknown, the results lose some of their value. The same criticism applies, with perhaps greater force, to the conclusions of Ory and his co-workers,[17] since they assume their samples of "commercial penicillin" to be G and proceed on the basis of this, probably unwarranted, assumption. These authors found all cocci tested, except staphylococcus, (*i.e.,* α- and β-streptococci, pneumococci, gonococci, and meningococci) to be two to eight times more sensitive to two samples of X penicillin (determined as 65% and >90% pure, respectively), than to 5 lots of "penicillin G." Three strains of *Klebsiella pneumoniae* and one of *Hemophilus influenzae* were equally resistant to both. The methods of testing were admitted by the authors to be crude, and the error was undoubtedly high. A more careful study of the comparative activities of X and G has been published by Libby and Holmberg.[18] Using some form of serial dilution test and highly purified preparations of G and X penicillins, they established that X is 2 to 4 times more active than G, in terms of staphylococcal international units, and 1.2 to 2 times as active, in terms of micrograms, against a variety of organisms. The importance of giving activities in terms of micrograms of penicillin, as well as in units, is, perhaps, never better illustrated than when the various penicillins are being compared.

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[11] **Abraham, E. P., & E. Chain.** Nature **146:** 837. 1940.
[12] **Holaday, H. A., & B. Shepherd.** Personal communication.
[13] **Veldee, M. V., R. P. Herwick, & R. D. Coghill.** Science **101:** 42. 1945.
[14] **Schmidt, W. H., G. E. Ward, & R. D. Coghill.** J. Bact. **49:** 411. 1945.

Some data have been presented on the antibacterial activity of X, as compared to G, *in vivo.*[16, 17] Thus, Welch and co-workers report the former to be 3 to 5 times as active as commercial penicillin against pneumococci Type I infection in mice, and more effective against gonorrhea in man;[16] while Ory and co-workers[17] found it slightly superior to "G penicillin," against gonorrhea in man. These results are open to the criticisms already given above, namely, the lack of information as to the composition of the "G or commercial penicillin" and the estimation of results in terms of staphylococcal international units, rather than in terms of weight (or, better yet, molar equivalents) of penicillin. The results of Ory and co-workers[17] also show that, at an equal dose in staphylococcal units, intramuscularly, higher levels and longer persistance are achieved in the blood serum with X, provided the tests are carried out with a streptococcus, against which X

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[15] **Rake, G., J. Fried, & O. Wintersteiner.** Unpublished data.
[16] **Welch, H., L. E. Putnam, W. A. Randall, & R. P. Herwick.** J. A. M. A. **126:** 1024. 1944.
[17] **Ory, E. M., M. Meads, & M. Finland.** J. A. M. A. **129:** 257. 1945.
[18] **Libby, R. L. & N. L. Holmberg.** Science **102:** 303. 1945.

is considerably more active, in terms of such staphylococcal units, than is G. When, however, 20,000 staphylococcal units of "G" were compared with 10,000 such units of X (*i.e.,* approximately the same amounts by weight of penicillin were given), the curves of potency obtained in the serum do not appear to be significantly different.

Determination of Activity

Apart from chemical methods for the detection of penicillin, details of which have hitherto been kept secret and use of which is only now coming to the fore, there are two chief biological methods of testing. These biological methods consist in measuring the antibacterial activity of the unknown preparation, under conditions identical with those used for testing a solution of a standard, of known activity in international units. The unitage of the unknown is derived from the comparison of its activity to that of the standard on the given day of testing. It is, thus, a reference unit; variations from day to day making any absolute unit, in terms of biological activity, wholly unreliable.

Biological tests for antibacterial activity are carried out by variant uses of either the agar diffusion or the broth dilution methods. In the first of these, a volume of solution of penicillin, in appropriate dilution (usually to approximate between 0.25 and 2.0 units per ml.), is applied, in some way or other (inside cylinders, on absorbent paper discs, etc.), to the surface of agar suitably sown with a susceptible organism. The penicillin diffuses out into the agar and, up to the limit of the diffusion range of a concentration which is still inhibitory for the test organism, prevents growth and produces a zone of clear agar, which can be measured. Comparison of zone sizes produced by the unknown with those obtained by the solution of the standard, under like testing conditions, allows one to estimate the potency in units. Even a superficial consideration of this technique, in whichever of its variants, will indicate its inherent possibilities for error and the need for most careful standardization.

The second method measures the activity of a solution of penicillin, appropriately diluted, at equal, and preferably narrow, increments, against a young culture of a susceptible organism, in suitable nutrient broth. The end-points of partial or complete inhibition of bacterial growth are read, after a stated interval of time, either by the naked eye or by one or another physical method for determination of degrees of opacity. Comparison of the end-point for the unknown, with the similar end-point achieved with the solution of standard for the same testing conditions, allows one to estimate the potency in units (and in terms of the organism employed). The inherent errors in this technique differ markedly from those in the diffusion method, but are, perhaps, somewhat less.

It is essential that the values, obtained by whatever variant is used, of either of the two above tests be considered in the light of their actual significance. This significance, statistically considered, will depend on the test employed, the number of replicates run, the accuracy of the determination of the standard, the type of samples being assayed, and even the laboratory running the tests.

Thus, the standard deviation, or the standard error derived from it, has shown, in our hands, the following values in the cup test and the dilution test (with steps of 10% increment)† (TABLE 2). These data mean that two-thirds of the test results with the given number of replicates will fall within the given range of deviation. The other results will show even greater deviations. However, it should be emphasized that the degree of accuracy of these results depends on the daily use of carefully standardized techniques, by highly trained technicians. In two other laboratories employing the cup test, but without as carefully standardized techniques, the standard error for 8 cups was ±10% and the standard deviation, ±26.5%. It must be presumed that the standard deviation for the dilution tests would be proportionately increased, in this laboratory. In other groups where testing conditions are even less satisfactory, the standard error for any given number of replicates will undoubtedly be still higher. Moreover, where the dilution intervals are even greater than 10%, going even as high as 50% (two-fold steps), the standard error becomes very high.

TABLE 2

Number of replicates	Cup test	Dilution test
1	±16.0%*	±8.9%*
3	±11.3%	±6.3%
5	±8.0%	±4.5%
8	±5.7%	±3.4%
16	±4.1%	±2.3%

* Standard deviation, for one replicate; standard error, for 3 or more.

The above discussion deals only with the standard error obtained when samples are adequate and concentrations of penicillin are reasonably high. In dilution tests, in our hands, in which small samples of body fluids with low concentrations of penicillin were assayed, the standard error for 3 replicate tests was ±25%. Moreover, since all of these values are not absolute, but rather relative, in terms of a standard of known potency run concurrently, the significance of the results is closely linked to the reliability of the standard values for a given set of tests. The results, in our hands, are based on the daily use of an adequate number of replicates of three standards, cross-checked, by several operators in each of at least three laboratories. However, where only one standard is run, and in few replicates, the errors thus involved will all contribute to the unreliability of the final assay figure for a given sample. In view of the above, it is of interest to note that, in one case, where the clinical investigators give some idea of the magnitude of the error involved in their data, they state it to be approximately ±30%.[19]

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† For the data used in this table we are indebted to Mr. R. Blue of the Biological Laboratories of E. R. Squibb & Sons.

Finally, one should draw attention to another error inherent in the testing of biological fluids, particularly sera: namely, the fact that such fluids, particularly at low dilution, vary from man to man, or from animal to animal, in the effect they have on the sensitivity or resistance of a given test organism. Under these conditions, unit values obtained by titration of penicillin in body fluid, against a standard in some other solvent, should be accepted with caution. It might be best to set up the penicillin standard in a sample of the body fluid in question obtained before therapy was commenced (when it is available), but even this is not above criticism.

II. TOXICITY

The data available on the toxicity of penicillin suffer under several shortcomings, some of which have been discussed above. Thus, the scarcity of material, particularly in the early days of the investigation of penicillin, makes much of the information little more than indicative. Very little work has been done with highly purified, and even less with pure, penicillin. As will be shown below, much of the reported toxicity of "penicillin" is due to the cation used in preparation of the salt or to impurities concentrated along with penicillin, from the fermentation broth, by the extraction process. In many cases, the unit values of penicillin injected have been calculated by methods of which the error is relatively great; in some, only the weight of "penicillin" injected is given; in others, only the unit value. Very rarely are both given. Yet, until pure penicillins are available for toxicity studies, it is desirable that the assay of toxicity be given both in units and in milligrams of material.

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[19] **Rantz, L. A., & W. M. M. Kirby.** J. Clin. Invest. **23**: 789. 1944.

Acute Toxicity

Despite these shortcomings, it became obvious, early, that "penicillin," even as available at that time, was of surprisingly low systemic or cellular toxicity.[1] It also became clear that the acute toxicity of "penicillin," after intravenous injection, decreased, as the potency increased.[20-24] TABLE 3 gives an indication, culled from various sources, of this decrease of toxicity with increasing potency. Investigations, at the Food and Drug Administration,[25] of many lots from different commercial producers show that, by and large, the toxic dose increases with increasing purity. A comparison of data on a large series of batches from a single producer shows the same trend. Thus, 42 batches, produced between July 1942 and June 1943, had potencies from 69 to 333 units per mg. These were tested, each in 5 mice, at 100,000 units per kg. Eighteen showed no toxicity, but in 24, there were 31 deaths on the first, and 14 on the second, days. One hundred consecutive batches, toward the end of 1945, were tested at 150,000 units per kg. No deaths occurred. The potencies ranged from 900 to 1200 units/mg., an average of 1087 units/mg.[6] It should be understood that all these figures apply to sodium salts of mixtures of penicillins, probably, for the most part, F and G. With such material, then, the observed acute toxicity depends largely, if not entirely, on the concomitant impurities.

TABLE 3

Potency units/mg.	End-point measured	Units/kg.	Reference
60	LD50	57,000	23
90	Maximum tolerated	70,000	22
181	LD50	280,600	6
200	LD50	355,400	21
325	Tolerated without reaction	325,000	20
400	LD50	675,000	23
460	LD50	635,000	6
656	LD50	1,050,000	24
790	LD50	1,000,000	6

Most of the inoculations for estimation of toxicity have been given by the intravenous route. Scanty data on the comparison of the lethal

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[20] **Florey, H. W., & M. A. Jennings.** Brit. J. Exp. Path. **23:** 120. 1942.

[21] **Hobby, G. L., K. Meyer, & E. Chaffee.** Proc. Soc. Exp. Biol. & Med. **50:** 285. 1942.

[22] **Hamre, D. M., G. Rake, C. M. McKee, & H. B. MacPhillamy.** Am. J. Med. Sci. **206:** 642. 1943.

[23] **Robinson, H. J.** J. Pharm. Exp. Ther. **77:** 70. 1943.

[24] **Welch, H., D. C. Grove, R. P. Davis, & A. C. Hunter.** Proc. Soc. Exp. Biol. & Med. **55:** 246. 1944.

[25] **Welch, H., C. W. Price, J. K. Nielsen, & A. C. Hunter.** J. Lab. Clin. Med. **29:** 809. 1944.

dose of "penicillin," by this route, with that obtained by subcutaneous injection of the same material,[26, 27] show that the intravenous route gives toxicities 2 to 2½ times as high.

Cations other than sodium have been used in the preparation of commercial "penicillin." Florey and Jennings[20] found calcium penicillin to be more toxic than sodium, and stated "that it would be inadvisable to use the calcium salt for therapeutic purposes." At the Squibb Institute,[28] we were able to show that a calcium salt of penicillin (160 units/mg.) was 6 times as toxic as a sodium salt (180 units/mg.) prepared from the same starting material. $CaCl_2$ was even more toxic in terms of calcium, due, probably, to its higher ionization. However, Herrell and Nichols[26] found that more potent calcium salts (Florey and Jennings had used material of only 50 units/mg.), even if they were more toxic than sodium salts, were, nevertheless, safe for therapeutic use by parenteral injection. Welch and his associates studied the effect of the cation more thoroughly.[24] Five salts of penicillin—sodium, ammonium, strontium, magnesium, and potassium—were prepared from a single master lot and investigated for their acute intravenous toxicity in mice. A calcium salt from another source, but of the same order of potency, was included in the study. At the same time, the acetates of these cations were studied for toxicity. On the basis of milliequivalents of the cation, the order of increasing toxicity was: Na, NH_4, Sr, Ca, Mg, and K. The same order was found for toxicity of the acetates. Relative toxicity, based on milligrams of cation at the L_D50, was: Na, Sr, NH_4, Ca, K, and Mg, in increasing toxicity; the same data, for the acetates, gave: Na, Sr, NH_4, K, Ca, and Mg. The authors conclude that "the toxicity of salts of penicillin is primarily due to the cations used in their preparation." In a second paper,[25] Welch and associates point out that, when sodium penicillin of low unitage is tested, the cation is not responsible for the toxicity; this is due, rather, to organic impurities concentrated in the final product by the extraction process. They also point out that, even with high potency preparations of magnesium penicillin, the toxicity of the Mg cation is such as to render inadvisable parenteral injection of such material in man.

Delayed Toxicity

Less study has been undertaken of the effect of repeated injections of "penicillin," but clinical results, in man, indicate that repeated injections of 10,000 units per kilogram per day, or higher, are tolerated without any systemic harmful effects, other than those due to sensitization, which are discussed below. In rabbits and mice, given repeated doses comparable to those commonly used in man (*i.e.*, 10,000 units/kg.), or 4 to 6 times higher, no evidence of toxicity was found.[22] However, a daily subcutaneous dose of as little as 7000 units/kg., in guinea pigs weighing between 175 and 355 grams, produced anorexia, ruffled fur, lethargy, loss of weight, and, finally, death. Death occurred, occasionally, on the second day, but most deaths were on the 3rd, 4th, or 5th days, with one as late as the 12th. Similar results were obtained with "penicillin" from a different source, and another laboratory obtained similar results with doses from 4000 to 20,000 units/kg., using penicillin from 4 different sources.[29] In our own hands, 1000 units/kg., given in divided doses, subcutaneously, were tolerated well by guinea pigs. There was some slight evidence, in these early experiments, that the results might be due to some impurity or impurities, since some animals survived on material of higher potency (1068 units/mg.). However, even at this potency (71% pure, the definition of potency for crystalline sodium penicillin G, at that time, being 1500 units/mg.), three out of five animals, receiving 7000 units/kg. per day, died, and the other two were sick, although they eventually recovered. Finally, when a preparation of crystalline sodium penicillin G was used, 3 guinea pigs, at a daily dose of 7000 units/kg., and 2, at 14,000 units/kg., all died.[30] This crystalline material contained at least 95% crystalline penicillin G, perhaps 4–5% of other penicillins, and less than 1% of impurities, other than penicillin.* The reason for this unusual behavior of guinea pigs, as compared to other animals, upon repeated injections of penicillin, is obscure. It could be demonstrated that it was not related to vitamin C metabolism.[22] It may be that one is dealing with another manifestation of sensitivity to penicillin. The guinea pig is peculiarly prone to sensitivity reactions, and the early onset of symptoms (*i.e.*, immediately following the second dose), which might seem to exclude sensitization, is exactly paralleled by observations in man.[31] The feeding habits of laboratory guinea pigs make them particularly prone to the introduction of mold spores into the nasal passages and lungs and, thus, to sensitization to mold products. Jones[32] showed that the lungs of guinea pigs, kept with dusty litter or food, showed 75% to 90% contamination, particularly with streptothrices and molds.

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[26] **Herrell, W. E., & D. R. Nichols.** Proc. Staff Meet. Mayo Clin. **18:** 313. 1943.
[27] **György, P., & P. C. Elmes.** Proc. Soc. Exp. Biol. & Med. **55:** 76. 1944.
[28] **Rake, G., C. M. McKee, D. M. Hamre, & C. L. Houck.** J. Immunol. **48:** 271. 1944.

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* Our thanks are due to Dr. Oskar Wintersteiner, of the Division of Organic Chemistry, The Squibb Institute for Medical Research, for supplying this material.

[29] **Heilman, F. R., & W. E. Herrell.** Proc. Staff Meet. Mayo Clin. **19:** 89. 1944.

[30] **Rake, G.** Unpublished data.

[31] **Lyons, C.** J. A. M. A. **123:** 1007. 1943.

[32] **Jones, F. S.** J. Exp. Med. **36:** 317. 1922.

Convulsive Action

Observations, during the early work on penicillin, demonstrated that penicillin had a convulsive and lethal action on mice infected, in the cerebral meninges, with the agent of *Lymphogranuloma venereum*.[33] The penicillin was given intraperitoneally, and convulsions followed immediately upon all therapeutic doses given more than 20 hours after intracerebral infection. Later investigations showed that the convulsions were due to a local concentration of penicillin in the inflamed meninges, since it never was observed after simple brain injury, or even after intracerebral infection until the meningitis was well advanced. Although the investigation indicated that some of the convulsant action might be due to impurities, qualitatively similar reactions were produced by crystalline sodium penicillin G. Rammelkamp and Keefer[34] showed that intrathecal injection of 10,000 units, in man, produced increased pressure, severe headache, vomiting, and pleocytosis. 5000 units gave slighter symptoms. Walker and Johnson[35] have shown that convulsions may be produced by injection of penicillin (commercial material from 7 different producers) into the cerebral cortex, in man (10,000 units), cats (1000 units), monkeys (500 units), and mice (20 units).

Local Reaction

No lesions are found in the organs of rabbits, guinea pigs, or mice receiving daily doses of penicillin, up to 60,000 units/kg., for 7 or more days, by the subcutaneous route,[22] nor have others described any lesions in the organs. Local changes are, however, produced at the site of repeated subcutaneous or intramuscular inoculation.[22, 36] To the naked eye, the local subcutaneous site appears edematous and hemorrhagic, and the intramuscular is often hemorrhagic. Microscopically, in addition to the edema and hemorrhage, there is an intense cellular infiltration, with monocytes and neutrophils predominating. If injection has been made into the subcutaneous tissue, the involvement, particularly in rabbits, where the inflammation is most intense, tends to spread to the subjacent muscle, with necrosis and phagocytosis of muscle fibers, and cellular infiltration. As might be expected, the local irritation and damage are related to the degree of purification, and much of these can be ascribed to the presence of impurities.[37] Thus, irritation and tissue destruction were more marked, after intramuscular injection of the same number of units of material with a potency of 186 units/mg., than of material with 588 units/mg.[36] As might be expected, the degree of damage is dependent on the concentration of penicillin. With material of potency better than 1000 units/mg., no difference was detected between calcium and sodium salts of penicillin.[36]

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[33] **Rake, G., & H. Jones.** Am. J. Syph. Gon. Ven. Dis. In press.
[34] **Rammelkamp, C. H., & C. S. Keefer.** Am. J. Med. Sci. **205:** 342. 1943.
[35] **Walker, A. E., & H. C. Johnson.** Arch. Surg. **50:** 69. 1945.
[36] **Linegar, C. R., & B. Thomas.** Personal communication.

It seems probable that changes, similar to those described in laboratory animals, occur in man after subcutaneous or intramuscular injection. Keefer and his associates[38] draw attention to the quite severe pain produced by the application of sodium "penicillin" to raw surfaces, and Florey and Florey,[39] confirming this, state that the calcium salt produces less pain. Both Keefer's committee[38] and Lyons[31] draw attention to the soreness or burning pain sometimes experienced at the site of local injection in some patients. Welch and Rostenberg[40] found that commercial sodium penicillin was a mild primary irritant, when injected intracutaneously, while crystalline penicillin (type not stated) was not.

Cytotoxicity

Commercial penicillin has a direct effect on cells, and cruder preparations are cytotoxic at higher, but not at lower, concentrations.[41] Rammelkamp and Keefer note that the cells evoked by intrathecal injection of penicillin are all motile and healthy.[34] It has been reported that crystalline sodium penicillin G can replace an osmotically equivalent amount of NaCl in Locke's solution, without affecting the oxygen consumption of various cells.[42]

Sensitization: Urticaria

That sensitization to penicillin could occur, was noted early. Three forms, in particular, have been observed: urticarial, tuberculin-like, and eczematous. The first occurs in some 3% of all individuals receiving penicillin by the usual parenteral routes (31 cases in 991 individuals receiving penicillin),[31, 38, 43, 44] or may even occur after local therapy, alone.[31, 45] The reaction is unusual in certain respects. It may be transient, even in the face of continued use of penicillin.[31, 38] It may appear, during the first 24 hours of treatment, after only one exposure, even in patients who have never before received penicillin,[31] and it may not recur when penicillin treatment is revived, after a lapse of time.[31, 38, 46] The urticaria may appear as an isolated phenomenon, but, in severe cases, the picture is one of "serum sickness," including pruritus,[46, 47] asthma,[47] dermatographia, lacrimation and sneezing, and eosinophilia.[31] Precipitin tests with the patients' serum are negative in some cases,[31] positive in others,[48] and the same is true with cutaneous tests for sensitivity, using commercial penicillin.[31, 46, 47, 48] In one case, the sensitivity to intradermal injection of penicillin was elicited with 3 brands of penicillin, but not with a fourth, suggesting that an impurity, or one particular type of penicillin, was responsible. Treatment was continued, with the 4th brand, without further reaction.[46] In another case, while skin tests with penicillin were positive, they were negative with extracts of *Penicillium notatum*. This case also gave a positive serum transfer test, but no anaphylactic antibodies could be demonstrated.[48]

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[37] **Herwick, R. P., H. Welch, L. E. Putnam, & A. M. Gamboa.** J. A. M. A. **127:** 74. 1945.

[38] **Keefer, C. S., F. G. Blake, E. K. Marshall, Jr., J. S. Lockwood, & W. B. Wood, Jr.** J. A. M. A. **122:** 1217. 1943.

[39] **Florey, M. E., & H. W. Florey.** Lancet **1:** 387. 1943.

[40] **Welch, H., & A. Rostenberg.** J. A. M. A. **126:** 10. 1944.

[41] **Abraham, E. P., E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley, M. A. Jennings, & H. W. Florey.** Lancet **2:** 177. 1941.

[42] **van Dyke, H. B.** Proc. Soc. Exp. Biol. & Med. **56:** 212. 1944.

[43] **Dawson, M. H., & G. L. Hobby.** J. A. M. A. **124:** 611. 1944.

[44] **Stokes, J. H., T. H. Sternberg, W. H. Schwartz, J. F. Mahoney, J. E. Moore, & W. B. Wood, Jr.** J. A. M. A. **126:** 73. 1944.

Sensitization: Tuberculin-Like Reactions

Tuberculin-like reactions have been studied by Welch and Rostenberg.[40, 49] They noted, during some studies on local reactions to impurities in penicillin, that one individual, with no previous contact with penicillin, but with a history of working with molds for 15 years, showed erythema and infiltration, with some vesiculation, coming on 6 hours after injection of 1000 units in 0.05 ml. intradermally, and reaching a peak after 72 hours.[40] This individual was later reinoculated with 1000 units, intradermally, of both crystalline and commercial penicillin. Two 'control' normal individuals were similarly injected, simultaneously. They showed no reaction, at any time, but the sensitive individual, though showing no reaction at 2 hours, reacted with 15 mm. areas of erythema and infiltration within 24 hours. A patch test with commercial penicillin was positive, when applied over areas previously injected with either crystalline or commercial penicillin, but not over previously uninoculated skin. Patch tests, with the medium used in penicillin production, were negative. No precipitin reactions

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[45] **Keyes, J. E. L.** J. A. M. A. **126:** 610. 1944.

[46] **Flinn, L. B., L. C. McGee, W. P. Featherstone, & D. O. Kern.** Delaware Med. J. **17:** 183. 1945.

[47] **Price, D. E., D. J. McNairy, & E. L. White.** J. A. M. A. **128:** 183. 1945.

[48] **Criep, L. H.** J. A. M. A. **126:** 429. 1944.

[49] **Rostenberg, A., Jr., & H. Welch.** Am. J. Med. Sci. **210:** 158. 1945.

were obtained with the patient's serum and no transfer of sensitivity could be obtained in man or rabbits.

In a subsequent paper,[49] the authors note tuberculin-like type of sensitivity in 8 of 144 individuals. Again, no passive transfer of sensitivity was possible. None of these individuals reacted to extracts of 6 species of fungi or to heat-killed spores of *Penicillium notatum.*

In 2 of 6 individuals receiving frequent intradermal injections of commercial penicillin, a flare reaction of old sites of injection was noted.[40] This did not occur after a rest period, but could be re-established by renewed, repeated injections. Such flare reactions could be induced best with injections given intradermally (not subcutaneously or intravenously), 5 days apart, and occurred with both crystalline and commercial penicillin.[49]

Sensitization: Eczematous Dermatitis

The third type of reaction has been noted in individuals who come into contact with penicillin by reason of their work in preparing, distributing, or administering the drug.[50–55] Here again, it occurs in approximately 3% of individuals exposed (9 of 312, in our own experience).[55] The lesions, which are usually eczematous in nature, affect, particularly, the face, hands and, in the male, the penis. They disappear when handling of penicillin is discontinued, and reappear on renewed contact. Patch tests with penicillin, whether crystalline or commercial, are usually positive, although in one case,[53] the test, while positive to commercial penicillin, was negative to a preparation of crystalline sodium salt. In some cases, dermatophytosis pedis is also present,[51, 52] but in others this is not noted. Among the reactions noted by Stokes and his collaborators,[44] was an "id" reaction, and Romansky[56] has noted the same in patients with dermatophytosis pedis. In two cases, sensitivity to intradermal injections of trichophytin was noted,[51] and in another case, of sensitivity following parenteral administration of penicillin, positive reactions were obtained on intradermal injection of both trichophytin and oidiomycin.[57]

It has been shown, therefore, that sensitization to penicillin can occur spontaneously or can be evoked. There are certain features of this sensitivity which are atypical, and many of these, as, for example, the

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[50] **Pyle, H. D., & H. Kattner.** J. A. M. A. **125:** 903. 1944.

[51] **Graves, W. N., C. C. Carpenter, & R. W. Unangst.** Arch. Derm. Syph. **50:** 6. 1944.

[52] **Binkley, G. W., & A. Brockmole.** Arch. Derm. Syph. **50:** 326. 1944.

[53] **Silvers, S. H.** Arch. Derm. Syph. **50:** 328. 1944.

[54] **Barker, A. N.** Lancet **248:** 177. 1945.

[55] **Eberlein, C. W., & G. Rake.** Unpublished data.

[56] **Romansky, M. J.** Personal communication. 1946.

[57] **Lamb, J. H.** Arch. Derm. Syph. **52:** 93. 1945.

apparent frequency of spontaneous desensitization, could be best explained on the assumption of a weak antibody response to the antigen or antigens responsible, so that this antibody is soon exhausted. Work of Chow and McKee[58] suggests that a loose combination, of uncertain nature, may be formed between crystalline penicillin and serum albumin. The formation of such a loose compound, if confirmed, might explain some of the phenomena of sensitization noted.

There is no clear evidence as to whether the sensitization is to penicillin itself, or to some of the impurities present in commercial penicillin. It may be that both are possible and occur in one or the other case. While sensitivity has been demonstrated with crystalline penicillin,[40, 49] in other cases it had been elicited with the commercial, but not with the crystalline, preparation,[53] or with three brands, but not a fourth, of "penicillin."[46] Moreover, crystalline penicillin is, almost certainly, usually not entirely pure, and the very small amounts of impurities present might sensitize, or elicit sensitivity reactions in already-sensitized individuals.

If the sensitivity is due to substances other than penicillin itself, the reactions on the first or second exposures to penicillin can be understood. Not only are a considerable number of people sensitive to penicillium antigen itself (6% in some areas of the mid-western states),[59] but, as has been shown for the penicillia[59] and the hyphomycetes,[60, 61] there is often marked cross-reaction within a group, suggesting a common antigen, perhaps of polysaccharide nature.[60] In some cases, cross-reactions between the hyphomycetes and the penicillia may occur,[51, 57, 62] which might account for the reactions to penicillin in individuals with dermatophytosis pedis. The fact that patients sensitive to penicillin have not been found sensitive to extracts[48] or heat-killed spores[49] of *Penicillium notatum*, or the reverse, does not necessarily speak against the hypothesis of common antigens as a source of sensitization. The matter of quantities may be involved, since, even in cases of known penicillin sensitivity, reactions were not induced in some individuals with a small dose of penicillin, but were with a larger one (*e.g.*, Rostenberg and Welch,[49] and others). The hypothesis of weak antibody response, suggested above to account for other of the unusual phenomena, would play a part in this connection.

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[58] **Chow, B. F., & C. M. McKee.** Science **101:** 67. 1945.
[59] **Feinberg, S. M.** J. Allergy **15:** 271. 1944.
[60] **Jadassohn, W., F. Schaaf, & M. B. Sulzberger.** Klin. Woch. **11:** 857. 1932.
[61] **Jadassohn, W., F. Schaaf, & G. Wohler.** J. Immun. **32:** 203. 1937.
[62] **Sulzberger, M. B., & F. S. Kerr.** J. Allergy **2:** 11. 1930.

Action on the Uterus

McClosky and Smith studied sensitization of guinea pigs with "penicillin."[63] They sensitized with 300 to 1000 units and, after an incubation period of one month, shocked with 8,350 to 23,640 units, intravenously or by intracardiac injection. Five animals were refractory, and two showed mild symptoms of a doubtful nature. It was also possible to elicit a positive response in the horns of the isolated uterus from sensitized guinea pigs, but this was not uniform. As a control to the latter, they showed that 30,000 units of "penicillin" had no appreciable effect on the contractions of the uterus from unsensitized animals. van Dyke[42] found that crystalline sodium penicillin G, at a final concentration of $1 \times 5.46 \times 10^{-3}$, produced a trivial contraction in an isolated virgin uterus from an unsensitized guinea pig. In humans, however, the administration of penicillin during pregnancy may produce signs of uterine activity or actual abortion.[64, 65] Thus, Lentz and his co-workers[64] note two cases of threatened abortion, appearing 18 or 48 hours after commencement of therapy, in pregnant syphilitic women (*i.e.*, when 100,000 or 300,000 units had been given). In Leavitt's series, 8 of 21 patients showed signs of uterine activity, and two aborted. Of these 8, 7 had only gonorrhea, and one had gonorrhea and syphilis. In most cases, only 50,000 units of commercial penicillin had been given. Since 7 of the 8 cases had received the same lot of penicillin, Leavitt believed that the increased uterine activity might be due to an impurity, and not to penicillin itself. He also believed that penicillin brought about a premature onset of menstruation.

There is one other toxic manifestation which may occur during penicillin therapy, and should be mentioned, although it has no relation to the toxicity of penicillin itself. This is the exacerbation of previous symptoms or appearance of new ones, of which the occurrence may be due to the rapid destruction of the infecting organism. It has been noted, for example, in the flare-up of staphylococcal infections of the skin, but is most apparent and severe in the treatment of syphilis[44, 65] (Herxheimer reaction).

Miscellaneous Pharmacological Action

There are remarkably few data on the general pharmacological actions of penicillin, chiefly because nothing of significance has been uncovered. Doses of commercial or pure G penicillin, in excess of those ordinarily used in therapy, have little effect, when given intravenously, on heart beat or respiration.[41, 42] The effect on blood pressure, like the acute toxicity, is due rather to the cation or the impurities present than to the penicillin. Thus, commercial sodium penicillin at 186 units/mg. gave the following depressions of blood pressure, after intravenous injection into rabbits: 10% with 35,000 units/kg., 40% with 55,000 units/kg., and 60% with 155,000 units/kg. With sodium salt at 233 units/kg., only a 10% drop occurred at 95,000 units/kg. When calcium salt was used, a preparation with 67 units/mg. gave a depressor effect at 20,000 units/kg., but calcium gluconate containing an equivalent amount of calcium gave a similar effect, and with calcium chloride the effect was much greater. Calcium salt with a potency of 895 units/mg. gave no depressor effect at 581,650 units/kg.[36]

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[63] **McClosky, W. T., & M. I. Smith.** Proc. Soc. Exp. Biol. & Med. **57:** 270. 1944.

[64] **Lentz, J. W., N. R. Ingraham, Jr., H. Beerman, & J. H. Stokes.** J. A. M. A. **126:** 408. 1944

[65] **Leavitt, H. M.** J. Ven. Dis. Inform. **26:** 150. 1945.

In all respects, therefore, penicillin is unique, not only in the wide range between therapeutic and toxic doses, but because of its almost negligible effect on mammalian protoplasm.

III. PHYSIOLOGICAL DISPOSITION

Data on absorption, distribution, and excretion are indispensable for the rational use of any drug. With penicillin, this is especially true, because of the need to maintain blood levels throughout the period of therapy. A great body of information as to the disposition of penicillin has now accumulated, but it is difficult to assess and correlate it, for reasons discussed above. It is highly probable that many of the apparently contradictory conclusions are due to differences in interpretation of, rather than to differences in, observations. Of the known pure salts of penicillin, only G and X, in relatively small amounts, have been available for studies in animals. There is little doubt that one of the greatest gaps in our knowledge of the pharmacology of the penicillins is that of the comparative behavior of the various pure preparations.

Absorption

It is well known that penicillin is absorbed rapidly, when injected intramuscularly or subcutaneously.[39, 66] Figure 3 illustrates a typical curve of blood and urine concentrations, such as occurs in dogs following a single subcutaneous injection.[67] Similar results are obtained in man. Maximal concentrations are produced in 15 to 30 minutes, but

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[66] **Rammelkamp, C. H., & C. S. Keefer.** J. Clin. Invest. **22:** 425. 1943.
[67] **Richardson, A. P., M. W. Ahlgren, & I. Miller.** Unpublished data.

maintained only for short periods of time. The rate of disappearance of penicillin from the blood is almost as rapid as its absorption. From the standpoint of effective chemotherapy of systemic diseases with penicillin, it is probably of greatest importance that blood and tissue fluid levels be maintained constantly above a certain minimal concentration.

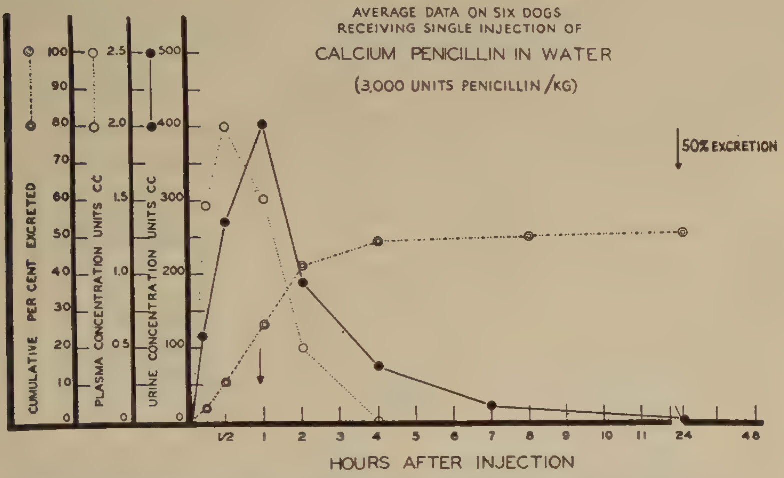

FIGURE 3. The excretion of calcium penicillin in water following subcutaneous injection. Each point represents the average determination obtained with six dogs.

Because of the rapid excretion, regimes of continuous or repeated administration must be used to obtain the greatest chemotherapeutic effect. Data such as those obtained by Rantz and Kirby[19] are, therefore, of great value, from a clinical standpoint. FIGURE 4, taken from their paper, summarizes their results. Continuous intravenous infusions of 100,000 units in 24 hours produced approximately 0.1 units/ml. of plasma; 200,000 units, approximately 0.2 units/ ml.; and 400,000 units, approximately 0.4 units/ml. Subcutaneous infusion produced somewhat lower levels. Anderson and others[68-71] found that continuous intramuscular or intravenous infusion produced comparable blood levels.

Studies on absorption of penicillin from the gastrointestinal tract were made, by Florey[41] *et al.*, soon after they prepared clinically usable material. They conclusively demonstrated that absorption would take place following oral administration in cats, rabbits, and man. These

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[68] **Anderson, D. G.** New Eng. J. Med. **232:** 400. 1945.
[69] **Hirsh, H. L., & H. F. Dowling.** Am. J. Med. Sci. **210:** 435. 1945.
[70] **Smith, R. O., & C. G. Harford.** J. Lab. Clin. Med. **30:** 502. 1945.
[71] **Loewe, L., P. Rosenblatt, M. Russell, & E. Alture-Werber.** J. Lab. Clin. Med. **30:** 730. 1945.

studies were later confirmed and extended, by Rammelkamp and Keefer.[66] Both groups of investigators pointed out that, since lower plasma levels and poor urinary recoveries resulted from oral, as compared with parenteral, administration, for the sake of economy only the latter route should be used. As penicillin became more plentiful, the problem of oral administration was reopened and has been extensively studied.[72–90] Unfortunately, it is difficult to draw conclusions from much of the work, because of lack of adequate controls.

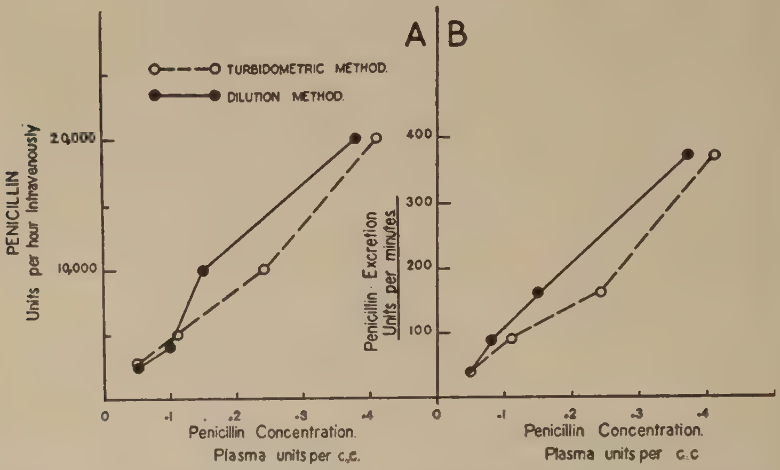

FIGURE 4. A. Relationship between amount of penicillin injected and plasma concentration. B. Relationship between rate of urinary excretion of penicillin and plasma concentration. Penicillin administered by continuous intravenous infusion.
(From **Rantz & Kirby.** J. Clin. Invest. **23:** 789. 1944.)

FIGURE 5 illustrates average results obtained following oral administration of commercial sodium penicillin in water, to four normal sub-

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[72] **Cutting, W. C., R. M. Halpern, E. H. Sultan, C. D. Armstrong, & C. L. Collins.** J. A. M. A. **129:** 425. 1945.
[73] **Ross, S., F. G. Burke, & P. A. McLendon.** J. A. M. A. **129:** 327. 1945.
[74] **Burke, F. G., & G. Strauss.** J. A. M. A. **128:** 83. 1945.
[75] **György, P., H. N. Vandegrift, W. Elias, L. G. Colio, F. M. Barry, & J. D. Fisher.** J. A. M. A. **127:** 639. 1945.
[76] **Shoemaker, W. G., & L. D. Seager.** Am. Prof. Pharmacist **11:** 613. 1945.
[77] **Paul, W. D., C. Rhomberg, & E. Wallace.** J. Indiana Med. Assn. **38:** 298. 1945.
[78] **Nelson, H. G.** J. Kansas Med. Soc. **43:** 224. 1945.
[79] **Seeberg, V. P., & M. F. Collen.** Science **102:** 225. 1945.
[80] **Perlstein, D., R. G. Kluener, & A. J. Liebmann.** Science **102:** 66. 1945.
[81] **Krantz, J. C., W. E. Evans, & J. G. McAlpine.** Science **101:** 618. 1945.
[82] **Barach, A. L., G. Garthwaite, E. T. Oppenheimer, J. Forman, & H. Osburg.** Science **102:** 247. 1945.
[83] **Free, A. H., J. R. Leonards, D. R. McCullagh, & B. E. Biro.** Science **100:** 431. 1944.
[84] **McDermott, W., P. A. Bunn, M. Benoit, R. DuBois, & W. Haynes.** Science **101:** 228. 1945.
[85] **Libby, R. L.** Science **101:** 178. 1945.
[86] **Charney, J., H. E. Alburn, & F. W. Bernhart.** Science **101:** 251. 1945.
[87] **Heatley, N. G.** Lancet **248:** 590. 1945.
[88] **Little, C. J. H., & G. Lumb.** Lancet **248:** 203. 1945.
[89] **Finland, M., M. Meads, & E. M. Ory.** J. A. M. A. **129:** 315. 1945.
[90] **Bunn, P. A., McDermott, Walsh, S. J. Hadley, & A. C. Carter.** J. A. M. A. **129:** 320. 1945.

jects, three hours after breakfast. A number of points are of interest: First, the rate of absorption is rapid. Peak plasma levels are obtained almost as soon, after oral administration, as after subcutaneous and intramuscular injection. This can only mean that absorption takes place high up in the gastrointestinal tract, and that penicillin either is not absorbed or is destroyed farther down. In all experiments in man, with oral therapy, the peak of absorption has been reached in approximately half an hour, and plasma levels are ordinarily maintained for not longer than two to four hours. As might be expected, concentrations in urine parallel those in blood. After a single administration, penicillin is found in the urine for as long as 7 hours, but seldom for a longer period of time, in appreciable concentrations.

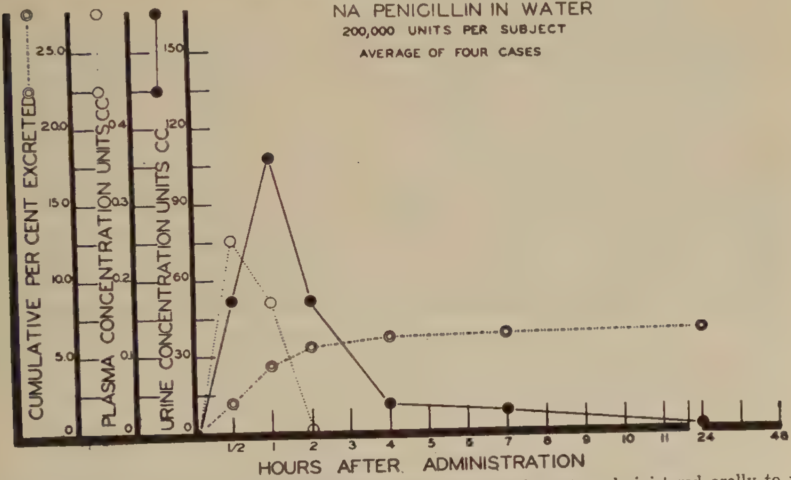

FIGURE 5. The absorption and excretion of sodium penicillin in water administered orally to four normal subjects. Each individual received 200,000 units, three hours after breakfast.

It is also characteristic that less than 10% of the dose administered by mouth can be recovered in the urine. In the four subjects illustrated in FIGURE 5, approximately 7% was recovered, and the greater portion of this was excreted in 1 to 2 hours. As pointed out later, about 50 to 75% of penicillin is excreted in the urine following parenteral injection. Assuming that, of the material which does get into the circulation, one-half is lost by processes at present obscure, it is reasonable to conclude that the 7.0% excreted actually represented only one-half of that absorbed. Thus, approximately 15% was absorbed in the oral experiments cited above, and one might expect that, if the orally administered dose were 5 to 6 times that used parenterally, comparable therapeutic results would be obtained.

Numerous attempts have been made to improve the absorption of penicillin from the gastrointestinal tract. There are three obvious possibilities, whereby penicillin might be destroyed, following oral administration: (1) acid gastric juice may inactivate it; (2) drainage from the gastrointestinal tract, passing first through the liver, may cause inactivation before the systemic circulation is reached; or (3) organisms present in the intestinal tract may destroy it, by production of penicillinase.

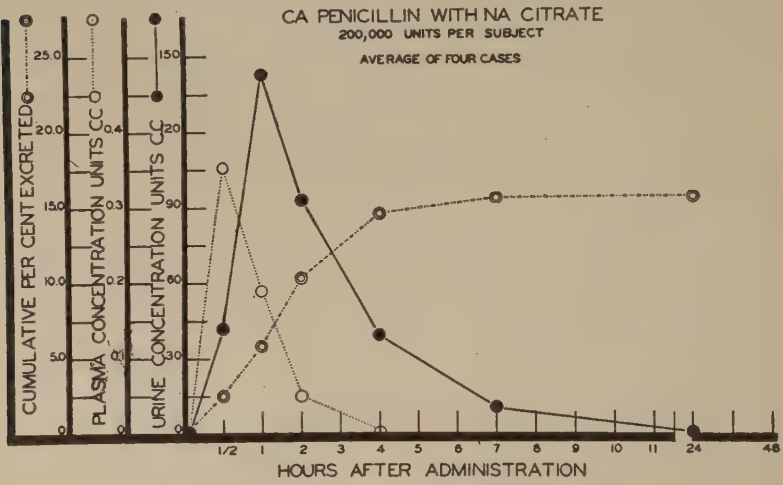

FIGURE 6. The absorption and excretion of calcium penicillin citrate tablets, after oral administration. Each tablet contained 20,000 units of calcium penicillin, and 0.5 grams of sodium citrate. Total dose per subject, 200,000 units. Administered three hours after breakfast.

Even in the earliest clinical studies, it was observed that the degree of acidity in the stomach played an important role in gastrointestinal absorption. Patients with achlorhydria absorbed considerably larger amounts of penicillin than did normal subjects, approximately 15% being recovered in the urine of patients with gastric acidity, as compared with 5 to 10%, for normal patients.[41, 91] There is some evidence against the supposition that penicillin may be destroyed in the liver. Thus, incubation of liver slices with penicillin *in vitro* leads to no loss in potency.[41] Moreover, injection of penicillin into the portal vein produces a plasma concentration equivalent to that obtained after a similar injection into the systemic circulation.[92] On the basis of present evidence, it is not possible to say whether poor gastrointestinal absorption is or is not due, in part, to destruction by penicillinase. One

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[91] **Rammelkamp, C. H., & J. C. Helm.** Proc. Soc. Exp. Biol. & Med. **54:** 324. 1943.

[92] **Cutting, W. C., F. P. Luduena, M. Fiese, H. W. Elliot, & J. Field.** J. Pharm. & Exp. Ther. **85:** 36. 1945.

may conclude, however, that the best means of increasing oral absorption is to protect penicillin, as it passes through the stomach and is in contact with the highly acid gastric contents. Since the evidence indicates that penicillin is absorbed in the upper part of the intestinal tract, one might expect that ordinary, enteric-coated capsules or tablets would serve little usefulness, and this is borne out in clinical trials. A number of investigators have suggested the administration of penicillin in oil or lanolin,[76, 80, 85] but, although absorption takes place from such vehicles, there is little evidence, at the present time, that such a procedure prevents destruction or improves absorption. The salts of penicillin are so water-soluble that it is unlikely that they would remain in the oil phase for more than a short period of time after mixing with gastric contents.

The only procedure which has given encouraging results is the administration of antacids, to neutralize gastric acidity. These have included sodium bicarbonate, sodium citrate, magnesium hydroxide, and various aluminum preparations. FIGURE 6 illustrates the degree of absorption of penicillin, when combined with sodium citrate, in the same four patients shown in FIGURE 5. Peak plasma levels are produced at about the same time after administration in both groups, and levels are maintained for a slightly longer period of time with citrate. Likewise, higher concentrations are found in the urine, and the amount recovered, in a 24 hour period, is approximately double that observed following administration of penicillin in water. Assuming that urinary recoveries account for only one-half of the absorbed penicillin, one may conclude that roughly 30% of the administered dose was absorbed. It still appears probable that, even under the best conditions, approximately three times as much penicillin would have to be given by mouth as by parenteral injection, to produce the same effects.

It has been suggested, by Welch and co-workers, that a single oral dose absorbed on aluminum hydroxide will maintain blood levels for 24 hours.[93] This has not been confirmed, either by our own work or by that of others. Moreover, Welch was unable to detect penicillin in the urine, after 7 hours. Such findings are contrary to most reported results, since concentrations in urine are always found to be many times that in blood. One is, therefore, tempted to question the validity of either the blood or urine assays reported by Welch and co-workers. Aside from these results, there is no evidence, at the present time, to indicate a special virtue of any particular antacid, and it is probable that the effectiveness of each is dependent, entirely, upon its ability to neutralize gastric contents.

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[93] **Welch, H., C. W. Price, & V. L. Chandler.** J. A. M. A. **128:** 845. 1945.

Oral administration of penicillin is also complicated by the relation of meals to degree of absorption. Usually, where this variable has been studied, less penicillin has been absorbed after meals than before.[74, 86]

Recently, attention has been paid to the administration of penicillin by the inhalation of penicillin-aerosol preparations.[94–101] The rate of absorption from the respiratory tract is rapid, peak concentrations in the blood being reached within an hour. Depending upon the procedure used, 5 to 20% of an administered dose may be recovered in the urine. Considering the loss of some penicillin in the exhaled air, this must mean that absorption from the lung is fairly satisfactory. Although aerosol inhalations have been proposed chiefly for local use in the respiratory tract, they might be used for systemic effect, under specialized conditions. It is not known, for certain, where, in the respiratory tract, penicillin is absorbed. From studies with other substances, one might infer that it occurs across the alveolar membrane or olfactory mucosa, and not from the mucous membrane of the larger air passages.

Because of the possible usefulness of penicillin in meningitis, and the difficulty of obtaining adequate concentrations in spinal fluids from parenteral injections, at least in the absence of inflammation, a number of investigators have studied the rate of absorption of penicillin from the spinal canal.[102, 103, 104] Absorption, here, is slower than from subcutaneous or intramuscular tissues, and detectable concentrations have been observed in the blood for as long as 7 to 8 hours. All the evidence available indicates that diffusion out of the spinal canal occurs at about the same rate as diffusion in, and varies with the degree of inflammation present.

Studies have also been carried out on the absorption of penicillin following injection into the pleura,[102-105] into knee joints,[106] into the

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[94] **Barach, A. L., F. H. Silberstein, E. T. Oppenheimer, T. Hunter, & M. Soroka.** Ann. Intern. Med. **22:** 485. 1945.

[95] **Olsen, A. M.** Proc. Staff Meet. Mayo Clinic **20:** 184. 1945.

[96] **Knott, F. A., & W. H. Clark.** Lancet **248:** 468. 1945.

[97] **Mutch, N., & R. E. Rewell.** Lancet **248:** 650. 1945.

[98] **Hanks, R. J.** Texas J. Med. **41:** 253. 1945.

[99] **Vermilye, H. N.** J. A. M. A. **129:** 250. 1945.

[100] **Hagen, E. W., M. Karp, & C. J. Farmer.** Arch. Otol. **41:** 333. 1945.

[101] **Romansky, M. J., D. J. Dugan, & G. E. Rittman.** Science **102:** 255. 1945.

[102] **Cooke, J. V., & D. Goldring.** J. A. M. A. **127:** 80. 1945.

[103] **Florey, M. E., & N. G. Healey.** Lancet **248:** 748. 1945.

[104] **Ory, E. M., M. Meads, B. Brown, C. Wilcox, & M. Finland.** J. Lab. Clin. Med. **30:** 809. 1945.

[105] **Fleming, A., M. Y. Young, J. Suchet, & A. J. E. Rowe.** Lancet **247:** 621. 1944.

[106] **Balboni, V. G., I. M. Shapiro, & D. M. Kydd.** Am. J. Med. Sci. **210:** 588. 1945.

chambers of the eye,[107, 108] or into other body cavities. In general, absorption has occurred more slowly than from subcutaneous or intramuscular injections. Although evidence is fragmentary, what is available indicates that penicillin penetrates poorly across uninflamed cellular membranes.

Excretion

Following parenteral injection, penicillin is found in urine and bile. However, by far the greater portion is found in urine, and, depending upon the conditions of the experiment, from 50 to 75% is ordinarily recovered following intravenous or intramuscular administration of an aqueous solution. The errors involved in the determination of penicillin in body fluids are such that it is difficult to obtain precise data on the mechanism of renal excretion. Rantz and Kirby,[19] and Jensen, Moller, and Overgaard[109] have, however, attempted to determine the plasma clearance of penicillin. The former found that, in man, from 755 to 1120 ccm. of plasma were cleared of penicillin per minute. The plasma clearance was not affected by the plasma level of penicillin or by the rate of urine formation. Similar results, of a much less satisfactory nature, have also been obtained in rabbits and dogs.[109] It has been repeatedly observed that renal failure[66, 110] or concurrent administration of diodrast[110] and para-aminohippuric acid[111–114] significantly delays the excretion of penicillin. In spite of the paucity of data, the only conclusion possible, at present, is that from 80 to 90% of the urinary penicillin is excreted by the renal tubules.

The data on excretion of penicillin in the bile are incomplete. Some is certainly excreted in this fluid, but the concentration in bile, as compared to that in plasma, varies considerably.[92, 115] In some experiments, bile-plasma concentration ratios approach unity, whereas in others, the ratio may be 4 or 5. Such differences may depend on whether the bile is derived from the gall bladder, where it has been concentrated, or directly from the primary biliary ducts. Some penicillin has also been found in the milk, and it is quite possible that

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[107] **von Sallmann, L.** Arch. Ophth. **34:** 195. 1945.

[108] **Leopold, I. H., & W. O. LaMotte.** Arch. Ophth. **33:** 43. 1945.

[109] **Jensen, K. A., K. O. Moller, & K. Overgaard.** Act. Pharm. et Toxicol. **1:** 184. 1945.

[110] **Humphrey, J. H.** Nature **154:** 765. 1944.

[111] **Beyer, K. H., H. Flippin, W. F. Verwey, & R. Woodward.** J. A. M. A. **126:** 1007. 1944.

[112] **Beyer, K. H., R. Woodward, L. Peters, W. F. Verwey, & P. A. Mattis.** Science **100:** 107. 1944.

[113] **Beyer, K., L. Peters, R. Woodward, & W. F. Verwey.** J. Pharm. **82:** 310. 1944.

[114] **Loewe, L., P. Rosenblatt, E. Alture-Werber, & M. Kozak.** Proc. Soc. Exp. Biol. & Med. **58:** 298. 1945.

[115] **Rammelkamp, C. H., & J. D. Helm.** Proc. Soc. Exp. Biol. & Med. 54: 31. 1943.

presence of penicillin in this body fluid comes about solely by diffusion from the blood.

Ordinarily, after intravenous injection, 75% of a single injected dose may be recovered in the urine. If absorption is delayed, as, for example, by the administration of penicillin-oil-beeswax mixtures, the percentage recovered in the urine is considerably less. The fate of penicillin which is not accounted for by urinary or biliary excretion is obscure. The incubation of penicillin with tissue slices results in little destruction.[41] Moreover, it has been shown that, in cases of anuria,[66, 110] detectable plasma levels are maintained for long periods of time.

Distribution

The high solubility in water and complete dissociation of salts of penicillin are determining factors in the ease with which this compound diffuses throughout aqueous media. Following injection into a body fluid, it distributes itself evenly throughout the total volume of that fluid. Precise measurements have not been made, but the plasma levels of penicillin, immediately following an intravenous injection, are such as to suggest that it is soon evenly distributed throughout the blood and extracellular tissues. For example, Rammelkamp and Keefer[66] report that the intravenous injection of 40,000 units, in man, produces a peak plasma concentration of 2.5 units per ccm. This indicates that the total volume in which penicillin is distributed is approximately 16 liters, which corresponds roughly to the total volume of blood and extracellular tissue fluids.

With the exception of the kidney, and probably the gastrointestinal tract, penicillin does not readily cross normal cellular membranes. Thus, in whole blood, less than 10% of that present is found in or on red cells.[66] Likewise, most studies on penetration of penicillin from the maternal blood stream to amniotic fluid or fetal blood show uniformly lower concentrations, in the fetus, when plasma levels of the mother are held constant.[116, 117, 118] The gastrointestinal tract probably represents a special case. In the upper tract, where absorption occurs, the intestinal contents probably have a low pH, and, under some conditions, penicillin is undoubtedly present as the free acid, rather than as the salt. As the free acid, penicillin is more lipoid soluble. Moreover, Frieden[120] has shown that, at low pH's, penicillin dif-

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[116] **Greene, H. J., & G. L. Hobby.** Proc. Soc. Exp. Biol. & Med. **57:** 282. 1944.
[117] **Hutter, A. M., & J. Parks.** Am. J. Obst. Gynec. **49:** 663. 1945.
[118] **Woltz, J. H. E., & H. A. Aintel.** Am. J. Obst. Gynec. **50:** 338. 1945.
[119] **Struble, G. G., & J. G. Bellows.** J. A. M. A. **125:** 685. 1944.
[120] **Frieden, E. H.** Science **101:** 21. 1945.

fuses more rapidly. Both of these factors may play a role in the absorption of penicillin, after oral administration.

Only meager data are available on the concentrations of penicillin in various tissues. In general, the results indicate high plasma-tissue concentration ratios.[92, 119]

Methods of Maintaining Plasma Concentrations

The rapid excretion of penicillin has complicated chemotherapy with this agent. Attempts to circumvent this disadvantage have been directed along two lines: (a) delaying excretion by the kidney, or (b) delaying absorption from the site of injection.

It has repeatedly been observed that patients with poor renal function, and, in particular, those with anuria, have prolonged blood levels, following the injection of single doses of penicillin. Furthermore, in nephrectomized animals, penicillin disappears from the blood slowly. A number of attempts have been made to delay the excretion of penicillin, in the course of therapeusis. These have usually been directed toward the administration of substances which modify tubular function of the kidney. Diodrast[110] and the aminohippuric accid[111-114] have both been used. Diodrast is too toxic for routine use, but Beyer *et al.* claim that a plasma level of 10 milligrams per cent of sodium p-aminohippurate doubles the peak concentration of penicillin in plasma, and delays the excretion in the urine. Although they have shown that this agent has low toxicity, it is not likely that it will receive widespread use, since it complicates therapy, and more effective measures are now available.

Moehlig and Linker,[121] and also Lich,[122] have reported that posterior pituitary extract will produce a prolonged concentration of penicillin in the blood. Presumably, this agent also acts, in part, by an effect upon tubular secretion. Bronfenbrenner and Favour[123] have obtained similar results, by restricting fluid intake and administering benzoic acid. All the methods proposed, thus far, for delaying excretion of penicillin by an effect on the kidney, are relatively ineffective. The best they can accomplish is to produce a higher level in the plasma and maintain a therapeutic concentration, for from 2 to 4 times that seen when penicillin alone is administered.

Romansky and Rittman[124] carried out experiments on absorption, in which penicillin was incorporated in peanut oil and beeswax. They proposed that the most suitable preparation should consist of 4.8% beeswax, in peanut oil containing 300,000 units of calcium penicillin per ccm. This concentration of beeswax was arrived at by testing preparations containing varying concentrations. We[67] have carried out experiments along this line in dogs. The same six animals were used throughout. Two hundred and fifty ccm. of water were administered at 9 a. m., and one hour later, each animal received, subcutaneously, 3,000 units of a calcium penicillin preparation. The blood levels and urinary

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[121] **Moehlig, R. C., & L. S. Linker.** Harper Hosp. Bull. **3:** 73. 1945.

[122] **Lich, R.** J. A. M. A. **128:** 1161. 1945.

[123] **Bronfenbrenner, J., & C. B. Favour.** Science **101:** 673. 1945.

[124] **Romansky, M. J., & G. E. Rittman.** Bull. U. S. Army Med. Dept. **81:** 43. 1944.

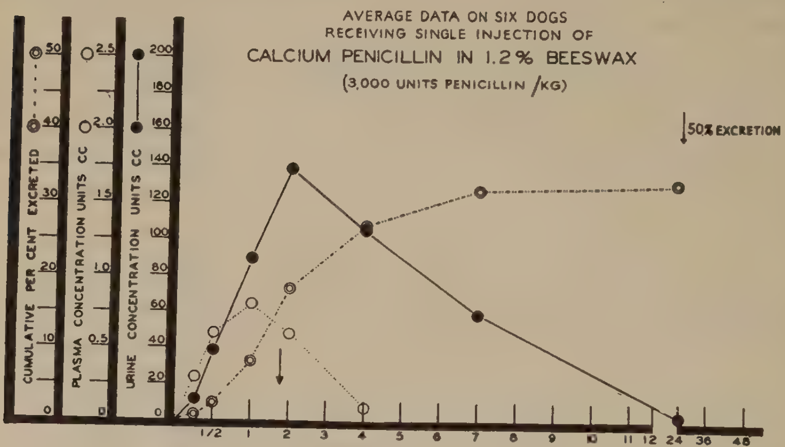

FIGURE 7.

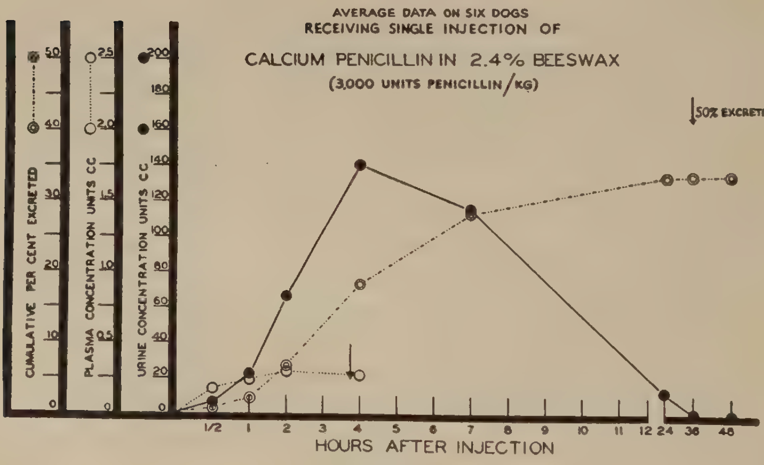

FIGURE 8.

excretion of penicillin administered in water, under such conditions, have already been discussed and described in FIGURE 3. In order to obtain some figure for basis of comparing the various preparations, we have arbitrarily calculated the time at which 50% of the total excreted penicillin was recovered in the urine, for the various preparations studied. FIGURES 7, 8, and 9 illustrate the effect of incorporating varying concentrations of beeswax into calcium penicillin-peanut oil mixtures. There was progressive delay in the peak of plasma concentration, and in the rate of excretion in the urine. The 50% excretion point, in either oil alone or in water, is about one hour, whereas, with 4.8% beeswax-peanut oil preparations, the 50% excretion point is delayed to about 7 hours. It is, obviously, impossible to transfer these results directly to man. We do feel, however, that the figure may be relative, and it can be assumed that blood levels may be maintained for approximately 7 times as long, with 4.8% beeswax-oil mixtures, as when aqueous solutions of penicillin are used. Similar results are obtained in man,[125–131] since it is usually accepted that therapeutic blood levels may be maintained from 8 to 12 hours, as compared with

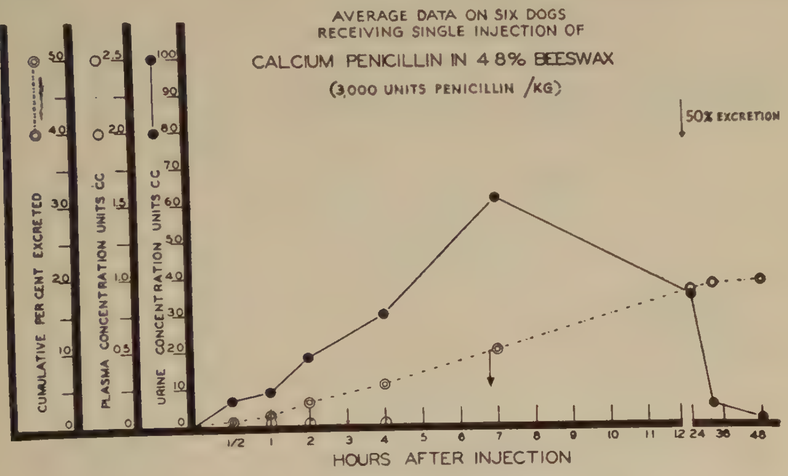

FIGURE 9.

FIGURES 7, 8, AND 9. The effect of varying concentrations of beeswax on the absorption of calcium penicillin from a peanut-oil suspension. Each preparation was studied in the same six dogs as those referred to in FIGURE 3.

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1 to 2 hours, when penicillin is injected in water. Romansky[125] and some others believe that adequate blood levels may be maintained for 24 hours.

We[67] have also carried out studies in an effort to determine which fraction of beeswax is responsible for the delayed absorption. U. S. P. white beeswax was extracted, according to the scheme illustrated by FIGURE 10, yielding 3 distinct fractions.* Each fraction was studied for its ability to delay the absorption of penicillin from peanut oil, according to the methods described above. A summary of these experiments, relating 50% excretion points to concentrations of each fraction, is presented in FIGURE 11.

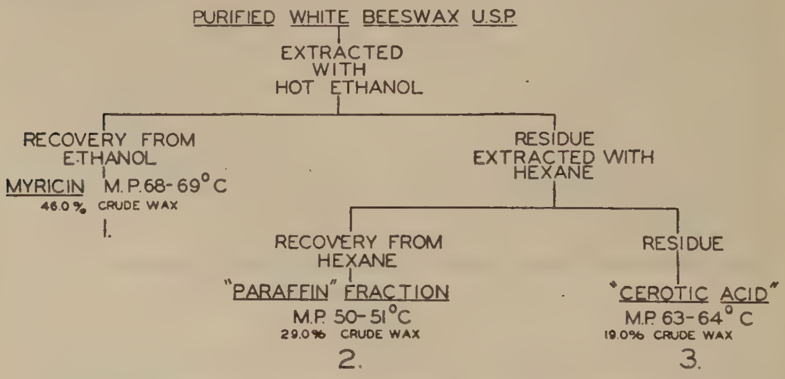

FIGURE 10. Method of separation of purified beeswax into various fractions.

It is apparent that each fraction possesses some ability to delay absorption. By far the most active fraction was myricin, which proved to be considerably more active than crude beeswax. Cerotic acid was approximately of the same activity as beeswax itself. The "paraffin" fraction was the least active, even the highest concentration used giving only slight delay in absorption. In general, there was good correlation between the melting point of the wax and its ability to delay absorption. A similar relationship has also been observed with a number of other waxes which have been studied. Only those having melting points of 60° C., or higher, appear to be promising.

A number of other methods have been used for delaying the absorption of penicillin. Various vasoconstrictors have been injected with penicillin, and prolonged blood levels have been obtained,[132, 133, 134] but only at doses which might produce systemic effects. At best, none of these procedures has produced as satisfactory results as has beeswax. Trumper and Hutter[135] have demonstrated prolonged absorption from the application of ice bags to the site of injection. They report maintaining therapeutic blood levels from 6 to 12 hours. Such a procedure may have usefulness, but certainly could not be applied widely in clinical practice.

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* We are indebted to Mr. W. A. Lott and Miss K. Losee of the Division of Medicinal Chemistry for these fractions.

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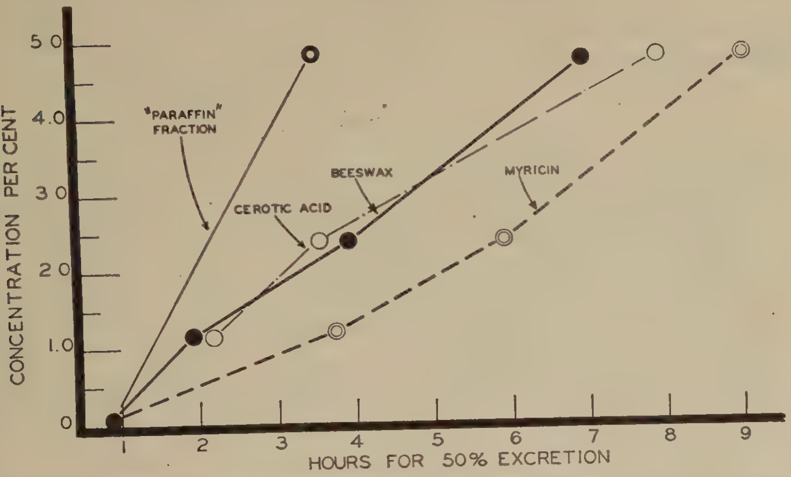

FIGURE 11. Summary of the effect of beeswax and its various fractions on the rate of absorption of calcium penicillin from a peanut-oil suspension. Horizontal axis represents time for excretion of 50 per cent of penicillin recovered in the urine. Vertical axis represents varying concentrations of beeswax or beeswax fractions.

Recent attention has been focused on the various esters of penicillin, because of their high fat and low water solubility. It was thought that, because of these properties, the esters might be absorbed more slowly from a subcutaneous or intramuscular injection than would aqueous solutions of the salts. Impure methyl, ethyl, butyl, and benzhydryl esters were prepared and studied by Meyer *et al.*,[9] who found them to be active in experimental bacterial infections in mice. Cavallito[10] *et al.*, more recently, prepared the benzyl ester from pure penicillin G and reported that it was more active than an aqueous solution of penicillin G, in the treatment of certain bacterial infections in mice. We have confirmed the observations of the above investigators, both in bacterial infections[136] and spirochetal infections in mice,[137] and have also been able to show that, in mice, the blood level of free penicillin in the plasma, following a subcutaneous injection of these esters, is comparable to that produced by the injection of an equivalent dose of one of the salts.[138] Unfortunately, the mouse appears to be unique in its ability to hydrolyze esters of penicillin, since we have been unable to detect appreciable free penicillin in the blood of monkeys, rabbits, dogs, or man, following an injection of the esters. More recently, Broh-Kahn and Smith[139] have reported that the butyl ester is hydrolyzed in mice, but not in man. Thus, it appears unlikely that any of the esters known, at present, will be of value in the treatment of human disease.

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ADDENDUM

Since this manuscript was prepared, several important preliminary papers have appeared which support the contention of the reviewers, that studies on the pharmacology of the pure species of penicillin are of extreme importance, from a practical and theoretical standpoint. Eagle and Musselman,[140] and Coghill *et al.*,[141] have both shown that, following injection of pure penicillin, F, K, G, or X, marked differences in plasma levels are obtained. With K, the plasma concentrations observed one hour after injection are much lower than those observed following injection of the three other species. Both groups of investigators conclude that K is rapidly destroyed in the intact animal and, therefore, relatively ineffective as a therapeutic agent. Whether such a conclusion is entirely justified, must await further study.

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STREPTOMYCIN IN TREATMENT OF CLINICAL AND EXPERIMENTAL TUBERCULOSIS

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It is appropriate to discuss streptomycin at this time, which represents the second anniversary of the announcement of the discovery of this remarkable antibiotic substance. The nature and properties of streptomycin were first described, two years ago this month, in the "Proceedings of the Society for Experimental Biology and Medicine," by Schatz, Bugie, and Waksman, from Dr. Waksman's laboratory, at Rutgers University. The first two lots supplied to us were produced in Dr. Waksman's research laboratory, and we are most grateful for the great effort which was required for its preparation. Subsequently, substantial amounts of streptomycin have generously been supplied to us by the research staffs of several pharmaceutical manufacturers, especially by Drs. Carlisle and Robertson, of Merck & Co.; Dr. George Hazel, of the Abbott Laboratories; and Dr. Gifford Upjohn, of The Upjohn Company.

Tuberculosis is a disease of such medical, social, and economic significance that great effort has been expended, for many years, in search for specific remedies. Many substances are capable of inhibiting or destroying the bacillus of tuberculosis *in vitro*. Robert Koch studied the action of compounds of gold and mercury on *Mycobacterium tuberculosis*, in 1890, and many unsuccessful efforts have since been made to apply this knowledge to treatment of experimental and clinical tuberculosis.[1] Unfortunately, the many synthetic chemical substances which were effective *in vitro* were found to be ineffective in treatment of experimental tuberculosis of guinea pigs. Promin was the first synthetic substance which was found[2, 3] to be capable of arresting the progress of well-established, experimental tuberculosis in guinea pigs. This encouraging discovery has resulted in a renewal of the chemotherapeutic attack against tuberculosis.

In addition to this prolonged study of synthetic compounds, which has occupied the attention of the last two generations of chemists and bacteriologists, there has been a parallel, but less intensive, study on the effect of antibiotic substances on *Mycobacterium tuberculosis*.[4]

Of the several antibiotic substances reported to be effective against *Mycobacterium tuberculosis, in vitro,* only one, streptomycin, has shown the ability to arrest well-developed tuberculosis, experimentally produced in guinea pigs.[5] Standardized and roughly quantitative methods have been developed,[6] which permit comparison of the results of treatment with streptomycin with those of previously studied synthetic compounds. Such studies have indicated that streptomycin is the most effective substance employed in treatment of experimental tuberculosis of guinea pigs.

Although streptomycin falls short of being the ideal remedy for treatment of experimental tuberculosis, it has been possible to show that prolonged treatment of guinea pigs, for a period of nearly six months, brought the disease to a state of apparent arrest more successfully than any of the previously described synthetic substances. After such prolonged treatment, lesions were resolved or suppressed in all of the animals. About 70 per cent of these animals still harbored viable tubercle bacilli and, in 30 per cent, there was reason to believe that the bacilli had actually been eliminated.

CLINICAL TUBERCULOSIS

The application of the foregoing facts to the treatment of clinical tuberculosis with streptomycin was promptly undertaken by us, and has now been under way for more than a year, during which time we have had the opportunity of treating sixty-three patients. We have included as many different types of tuberculosis as possible, in this study, in the hope of gaining information which would be of value in the guidance of more extensive and more crucial, subsequent, clinical studies. The experience of ourselves and colleagues, in treatment of tuberculosis, up to December, 1945, is summarized in TABLE 1. The criteria on which the opinion of favorable effects was based will require explanation, in each instance.

Five patients who had miliary tuberculosis have been treated. One of these survived only long enough to permit treatment for five days. This case should be clearly classified as a failure, because the drug was given in maximal amounts, and examination at necropsy revealed that the disease was in an early stage of development. Such a stage of development would appear to be a favorable time for treatment, if the drug has adequate therapeutic potential to cope with the situation. The other four patients were treated for periods ranging from two to four months, but despite what was apparently maximal treatment, all of these patients died. At necropsy, definite and usually striking evidence of healing was observed, especially in the lesions in the lung.[7] Serial roentgenographic examinations of the chest, in each instance, confirmed the belief that the pulmonary lesions of miliary tuberculosis underwent modification, such as we have not observed in any previous similar cases. These cases will be reported, in adequate detail, in the near future.

TABLE 1

TUBERCULOSIS TREATED WITH STREPTOMYCIN
(To January, 1946)

Type or site of tuberculosis	Cases	Apparent favorable response	Remarks
Miliary	5	4	Mortality 100 per cent; meningitis in 3 cases.
Pulmonary	22	17	Incomplete treatment in 2 cases; no change in 2; 1 death.
Tracheobronchial	3	3	Parenteral and aerosol therapy.
Empyema	6	0	Arrest with oleothorax later in 4 cases.
Sinus tracts	7	6	Marked tendency to recurrence; improved but not cured.
Renal	11	8	Marked tendency to recurrence; apparently arrested in 2 cases only.
Cutaneous	4	3	Temporary and partial improvement only.
Miscellaneous	5	4	
Total	63	45	No lesions progressed during treatment (except lesions of the central nervous system).

The twenty-two patients* who had pulmonary tuberculosis were selected with great care, from several hundred candidates for such treatment. In all cases selected, the disease had progressed unmistakably during the control period of observation, prior to institution of treatment. In each instance, it was believed that the disease would progress further, if treatment were not instituted. These patients are not representative of the usual types of pulmonary tuberculosis, but are highly selected individuals with exudative lesions, who appeared to lack normal ability to control the disease. Sufficient time has not elapsed, in most instances, to know whether the disease will be brought to a state of permanent arrest, as a result of treatment with streptomycin.

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* These include patients treated in collaboration with Dr. Karl Pfuetze, Mineral Springs Sanatorium, Cannon Falls, Minnesota.

It can be said, however, that in no instance did the disease continue to progress, so long as treatment with streptomycin was being administered. It also can be stated that the majority of these patients began to show clinical and roentgenographic evidence of slow, but definite, improvement, within four to eight weeks after institution of treatment. In many cases, active tuberculosis is still present, after two to five months of treatment. In many cases, also, the sputum remains positive and the prognosis still appears grave; in some, it is doubtful whether permanent benefit has been accomplished in those cases temporarily improved.

The seven patients who had external tuberculous draining sinuses include five patients with sinuses related to tuberculous lymphadenitis and two who had tuberculous involvement of the thoracic wall. In each instance, there had been no tendency to spontaneous closure of these sinuses before treatment. They had remained open, for periods varying from six months to two years. In each adequately treated patient of this small series, marked improvement, with at least temporary cessation of drainage, was observed within four to eight weeks after institution of treatment. Frequently, the presence of enlarged, palpable, tuberculous lymph nodes could still be noted after treatment. Already, three of these patients have had recurrent draining sinuses, since discontinuation of treatment; in one of these three, the sinuses later closed spontaneously; and in the other two, they closed after a second course of treatment with streptomycin.

Eleven patients who had renal tuberculosis* have been treated with streptomycin and are still under observation. It may be said that some symptomatic evidence of improvement was observed, in most cases, and in the few cases in which cystoscopy was carried out before and after treatment, there was objective evidence of healing of the ulcers in the bladder. Tubercle bacilli in the urine of all patients who received treatment for two months or longer were sufficiently reduced in number to make it difficult, or impossible, to detect their presence by conventional acid-fast methods of staining. However, bacteriologic observations are incomplete, and the results of guinea pig inoculation cannot be recorded, at this time, for the majority of patients. It may be said, however, that definite recurrence of bacilluria and recurrence of symptoms of vesical irritation have developed, within two or three months after cessation of treatment, in three of the five patients whose treatment was undertaken prior to August, 1945.

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* Treated in collaboration with Drs. E. N. Cook and L. F. Greene, of the Section of Urology.

Empyema of the pleural cavity would appear to be an ideal type of tuberculous infection to treat with streptomycin. However, experience in the treatment of six cases, in collaboration with Dr. Pfuetze, has been disappointing, so far. The pleural cavity was frequently intolerant, either to the injections of streptomycin or to impurities contained in the preparations used. Furthermore, it was not possible to bring about significant improvement, even with prolonged local treatment, which consisted of injection of streptomycin into the pleural cavity. Recently, we have noted that the hydrogen ion concentration of the purulent exudate in the pleural cavity is likely to be distinctly on the acid side. Since streptomycin is not active in acid media, it seems possible that the acidity of the exudate may have contributed to the failure of treatment with streptomycin, under these circumstances.

The small series of three cases of tracheobronchial tuberculosis is not sufficiently large to permit generalization. However, visible lesions in the tracheobronchial tree have healed with sufficient rapidity, following combined use of intramuscular injections of streptomycin and treatment with streptomycin aerosol, to urge strongly that more patients of this type be treated, when the drug becomes available. The aerosol therapy was carried out in the same manner as described for treatment of bronchiectasis.[8]

The miscellaneous group of cases includes tuberculosis of the larynx, tuberculous ulcers of the hypopharynx, tuberculous iritis, synovial tuberculosis of the knee joint, and a tuberculous infection in a wound of the scalp, following exploration for tuberculoma of the brain. Each of these patients improved rather promptly, after institution of treatment. The case of ocular tuberculosis could not be classified as one in which the favorable response was due solely to streptomycin, because of the fact that the batch of streptomycin used in treatment, in this case, produced a febrile reaction, and ocular tuberculous lesions frequently heal after nonspecific fever therapy.

Until streptomycin is available in greater quantity, it will be necessary to select with great care all candidates for treatment with it. This care in selection is especially necessary for those candidates who have tuberculosis, because of the relatively large quantities of the drug required for adequate treatment of this condition. We have regarded 1.0 to 2.0 gm. of streptomycin per day as the minimal dose, in most types of tuberculosis, and sixty days as a minimal course of treatment. The following principles are suggested to aid in selection of cases.

SELECTION OF CASES FOR TREATMENT

Streptomycin is not indicated for treatment of pulmonary tuberculosis which is minimal in extent. It is not indicated, at this time, for treatment of any parenchymal pulmonary lesion which is suitable for collapse therapy and in which satisfactory collapse can be produced. It is not recommended for treatment of lesions which are making satisfactory improvement on a therapeutic program of conventional type. Streptomycin should not be used as a substitute for known, proved, effective procedures. Streptomycin should not be expended for treatment of stable, arrested or chronic, nonprogressive types of pulmonary tuberculosis.

Treatment with streptomycin, in tuberculosis, is still an experimental procedure and should be restricted to those types of cases and to those situations in which a maximal amount of information can be obtained for guidance of subsequent trials with the drug. The clearest indication for use of streptomycin is in treatment of recent extensions of pulmonary tuberculosis to regions not previously involved, which have occurred despite sanatorium regimen. The indication is stronger, if the spread be extensive, and especially if it involves the contralateral lung which was not previously involved. The treatment appears to be more logical, if the infiltration appears to be finely divided on roentgenographic examination, than if the shadows be confluent, dense, and opaque.

In extrapulmonary tuberculosis, use of the drug should be restricted to bacteriologically proved cases and to those in which clinical improvement has not been satisfactory, with conventional methods of treatment. It does not appear logical, at this time, to combine streptomycin with any other type of treatment, such as surgery, which might yield satisfactory results alone.

COMMENT

Present experience indicates, rather clearly, that the antibacterial effects of streptomycin in clinical tuberculosis are limited to a suppressive action, which appears to retard the disease process. We have not yet determined whether this retarding effect can be maintained for a prolonged period of time or whether the development of drug-fast strains[9] will later nullify this apparent therapeutic effect. It may be stated that, as yet, definitely progressive lesions of pulmonary tuberculosis have not developed in any patient under our observation, while treatment with streptomycin was being used. Unfortunately, streptomycin does not appear to possess any rapidly curative action in tuberculosis, such as might resemble the therapeutic marvels achieved by several antibacterial drugs against some acute infectious diseases.

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SOME CONSIDERATIONS OF THE CLINICAL APPLICATION OF STREPTOMYCIN

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An evaluation of the therapeutic efficiency of streptomycin in the treatment of surgical infections was begun at the Halloran General Hospital in June, 1945. The study included three types of cases: acute and chronic infections in battle wounds, genito-urinary infections, and bacteriemias. The aerobic, pathogenic bacteria, causal of these infections, and their sensitivity in broth[1] to streptomycin, are listed in TABLE 1. The table reveals that streptomycin inhibits the growth of most pathogenic gram-positive and gram-negative bacteria. However, both groups manifest a wide variation in sensitivity. Of the gram-

TABLE 1

STREPTOMYCIN SENSITIVITY OF AEROBIC PATHOGENIC BACTERIAL FLORA OF 81 SURGICAL INFECTIONS

Gram-Negative organism Streptomycin u/cc.	0.5	1	2	4	8	16	32	64	128	Total
A. aerogenes	—	2	6	3	4	5	2	3	—	25
E. coli	—	1	5	6	5	2	2	—	—	21
Paracolon group	—	—	2	3	1	—	—	—	—	6
K. pneumoniae, Type A	—	—	4	3	2	—	—	—	—	9
K. pneumoniae, Type B	—	1	5	3	2	2	—	1	—	14
Proteus vulgaris	—	—	5	9	22	17	2	3	—	58
Proteus morganii	—	—	—	—	1	—	—	—	1	2
Ps. aeruginosa	—	—	—	—	7	11	3	—	4	25
Totals	—	4	27	27	44	37	9	7	5	160
Gram-Positive organism										
Staph. aureus, hem.	6	10	5	3	—	—	1	—	3	28
Staph. aureus, non-hem.	5	3	—	—	—	—	—	—	2	10
Strep. alpha, viridans	5	1	2	5	2	5	3	—	—	23
Strep. gamma, non-hem.	3	—	—	9	11	13	4	—	1	41
Strep. hem. beta	—	—	—	3	2	2	—	—	—	7
Totals	19	14	7	20	15	20	8	—	6	109
Grand Totals	19	18	34	47	59	57	17	7	11	269

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* Captain, M.C., A.U.S.
† Major, M.C., A.U.S.

negative organisms, the Colon and Friedlander groups, for the most part, are inhibited by 8 units or less of streptomycin. *Bacillus proteus, Aerobacter aerogenes,* and *Pseudomonas aeruginosa* are found to number many strains which require more than 8 units per ccm. for bacteriostasis. Of the gram-positive bacteria, staphylococci usually are inhibited by 8 units or less of streptomycin, whereas streptococci, regardless of their hemolytic properties, tend to be more resistant.

With any chemotherapeutic agent, it is important to know if it is checked in its action by body fluids. In TABLE 2, the *in vitro* action of some gram-positive and gram-negative bacteria is compared in F.D.A.* test broth with the same medium containing human citrated blood. The gram-positive and the gram-negative bacilli show little variation in their susceptibility in either of the two media, whereas non-hemolytic streptococci and hemolytic staphylococci require from 4 to 8 times more streptomycin to check their growth in the blood broth. Comparable results are obtained if human serum or plasma is substituted for whole blood. Thus, streptomycin activity is lessened against the gram-positive cocci in these body fluids.

TABLE 2

COMPARISON OF STREPTOMYCIN SENSITIVITY IN PLAIN MODIFIED AND BLOOD ENRICHED F.D.A. BROTH

Organism	F.D.A.	F.D.A. 3% Blood
B. subtilis	<0.5	1
B. anthracis	<0.5	<0.5
B. anthracis	<0.5	1
Staph. aureus	2	8
Staph. aureus "SM"	0.5	4
Strep. gamma Du	8	64
Strep. gamma Ca	8	64
Aerogenes-Fried.	64	64
Proteus vulgaris	4	8
E. coli	8	4
Ps. pyocyaneus	64	64
E. typhosa	2	2

These findings have obvious therapeutic implications. They raise the question of the comparative susceptibility of gram-positive cocci to streptomycin and to penicillin, also of the feasibility of combining the two agents. In TABLE 3 are listed the sensitivities of a collection of gram-positive cocci to streptomycin and to penicillin. It will be noted that no correlation exists between the two antibiotics. The preponderance of penicillin-resistant cocci is explained by the fact that the patients from whose wounds they were isolated had had prolonged courses of penicillin therapy. The findings suggest that the mechanism of action of streptomycin differs from that of penicillin. Only 5 of the 49 organisms were resistant to both penicillin and streptomycin. The practical importance of this finding is the implication that streptomycin may be of therapeutic value in infections due to penicillin-resistant cocci. Furthermore, we have found that subinhibitory amounts of streptomycin and penicillin, when combined, are additive in their action. Therapeutic advantage might, therefore, be gained by giving the two drugs simultaneously, if indicated by the clinical and laboratory findings. This would combine the additive effect of the two drugs, together with the greater effectiveness of penicillin against gram-positive cocci in the presence of blood.

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* Food and Drug Administration.

TABLE 3

COMPARISON OF *In Vitro* ACTION OF STREPTOMYCIN AND PENICILLIN ON GRAM-POSITIVE COCCI

rganism and number of strains	Streptomycin sensitivity "S" u/cc	Penicillin sensitivities Oxford u/cc								
		.019u/cc	.039	.078	.156	.312	.625	1.25	2.50	2.5+
taph. albus	(1) .5				+					
" "	(1) 128									+
taph. aureus non-hem.	(2) .5			+	+					
" "	(3) 1				+		+			+
" "	(1) 128					+				
taph. aureus hemolytic.	(3) .5			"SM"	+					+
" "	(10) 1	+	+	++	++	+				+++
" "	(4) 2				+					+++
" "	(4) 4									++++
" "	(1) 8									+
" "	(1) 128								+	
" "	(2) 128								+	+
trep. viridans alpha	(1) 2									+
" "	(1) 8									+
" "	(2) 16									++
" "	(3) 32									+++
" "	(1) 128			+						
Strep. hem. beta	(1) 8									+
" "	(2) 16									++
Strep. non-hem. gamma	(2) 16									++
" "	(1) 32									+
" "	(2) 128									++

Data presented in TABLE 1 indicate that, if the aerobic pathogenic bacteria of surgical infections are to be effectively controlled, levels of 8 or more units per ccm. of streptomycin must be maintained in the blood. A group of patients was given 400,000 units (0.4 gm.) of streptomycin, intramuscularly, every four hours. Blood serum levels determined on specimens drawn immediately prior to a scheduled dose revealed 8 to 16 units per ccm., 16 units being more usual after the first 6 doses of streptomycin had been given (TABLE 4). In the urine, levels of more than 500 units per ccm. were common, provided renal function was not impaired. The concentrations of the drug in the urine were considerably higher if the urinary output was restricted by limiting the fluid intake.

TABLE 4

STREPTOMYCIN BLOOD AND URINE LEVELS OF PATIENTS RECEIVING 400,000 UNITS I.M. EVERY 4 HOURS

Case	Blood, Serum	Urine
4	4–16 u/cc	168–1170 u/cc
6	8–16	384–1178
7	8–16	413–1190
9	8–16	448– 896
11	4–8	435–1177
13	4–16	819–1229
5	8–16	74– 221*
15	16	2000 plus **

* Practically non-functioning kidney (diodrast).
** Fluid intake restricted to 2500 ccm./24 hours.

BATTLE WOUND INFECTIONS

This dosage scheme has been adopted for the treatment of surgical infections. In addition, in some cases, local application of streptomycin, in solution, or in an ointment base containing 5,000 units per gram, has been employed. The group thus treated consists of 36 patients, the majority of whom had chronic osteomyelitis. The others were chronic and acute soft part or sinus tract infections.

Illustrative Case Reports

Case 1: A 27 year old soldier sustained, on 19 November, 1944, a gunshot wound of the lower third of the left leg, with fracture of both bones, and subsequent osteomyelitis. Two sequestrectomies were done: one on 24 May, 1945, and the other on 24 October, 1945. The patient had several courses of sulfadiazine as well as of penicillin, but the progress of the infection was unchecked. Cultures showed a hemolytic *Staphylococcus aureus, Paracolon bacillus,* and *Bacillus subtilis,* which were sensitive to 1, 4, and 8 units per ccm. of streptomycin, respectively. On 5 November, two weeks after the second sequestrectomy, he was given streptomycin intramuscularly, 400,000 units, every four hours for 4 days. In addition, the drug was applied locally in an ointment base containing 5,000 units per gram. Parenteral streptomycin was discontinued on the fifth day, because of nausea and vomiting. Local administration of streptomycin was continued. The bacteria disappeared from the wound, within the first week, and uncomplicated wound healing was achieved three weeks later. To date, there is no clinical or roentgenological evidence of bacterial activity.

Comment: This case may be considered a streptomycin success, as an adjuvant to surgery, in apparently controlling the infection.

Case 2: A 22 year old soldier was shot in the right lower abdomen, 17 May, 1945, with the wound of exit over the left iliac crest. He arrived at the Halloran General Hospital, 15 August, with a left inguinal colostomy, and a sinus opening in the left flank, which was draining profuse amounts of foul-smelling pus. Study of the tract by lipiodol visualization showed an extensive osteomyelitis of the 5th lumbar vertebra, penetration of the joint space between L5 and S-1, and involvement of the sacrum. He had lost 80 pounds of weight since the injury. Culture of the pus showed *Pseudomonas aeruginosa, Bacillus proteus, Staphylococcus aureus,* anaerobic hemolytic streptococci, and *Clostridium sporogenes.* The aerobic organisms were sensitive to 16, 16, and 2 units per ccm., respectively, of streptomycin. He was given 400,000 units of streptomycin intramuscularly every four hours, starting 13 October, for 10 days, in addition to streptomycin solution (5,000 units per ccm.) instillations and infusions into the sinus tract. Blood serum levels of 8 to 16 units per ccm. were maintained. No improvement resulted. While under treatment, he passed several small sequestrae through the sinus tract.

Comment: This case emphasizes the need for surgical cleaning-out of necrotic bone and thick sinuses, to render the organisms accessible to streptomycin. Dead tissue is non-sterilizable. The case illustrates the futility of employing streptomycin in infections where necrotic tissue is present and the blood supply is inadequate.

Case 3: A 27 year old white male, on 14 April, 1945, sustained a penetrating shrapnel wound of the right lower abdomen, a simple fracture of the right lower three ribs and paralysis of the left femoral nerve. He entered the "Streptomycin Ward," 24 September, for recurrent infection in the left groin, associated with imbedded shell fragments, culture of the wound showing *Bacillus proteus, Staphylococcus aureus,* and other organisms. There was drainage from an incisional wound in the left thigh. The infection had not been eliminated by previous penicillin, sulfadiazine, and surgery. Because of the apparent localization of the infectious process, local streptomycin therapy was tried first. Daily instillations of streptomycin solution or ointment resulted in progressive clinical improvement, but without sterilization of the wound. By 24 October, the wound was "healed." Two days later, however, the patient had a chill with fever and complained of diffuse pain in the left hip, which localized later in the groin. On 28 October, he was started on a 4-day course of streptomycin, intramuscularly, 400,000 units every four hours. On the second day of therapy, a deep abscess erupted through the wound scar and drained profusely for two days. Local streptomycin was continued in the wound after the systemic drug was discontinued. The patient again improved steadily, until the 8th of November, 11 days later, when another inflammatory reaction with fever, malaise, and pain occurred. A second abscess formed and was drained. The drug was given parenterally once more, and was continued for 8 days. This time, sterilization of the wound was achieved and the wound is healed, with no recurrence to date.

Comment: This case illustrates the failure of local therapy to contact all the reaches of a complicated wound infection, and the failure of a short course of systemic therapy to achieve a successful result. It also serves to emphasize that, in these chronic infections with tissue damage, foreign bodies, and mixed bacterial flora, it is necessary to give large doses of streptomycin for a long period of time. An interesting feature is the observation that the bacteria did not become drug-fast.

Case 4: This patient, on 7 February, 1945, sustained a penetrating mid-thigh wound resulting in foreign bodies in the hip joint, compound comminuted fractures of the head of the femur, septic arthritis of the hip joint, and retroperitoneal infection. At the time of presentation for streptomycin treatment, 26 September, 1945, he had drainage from the right ear, from sinuses in the median cystostomy wound, and from the exploratory scar over the lateral aspect of the right hip, which had failed to respond to local and systemic penicillin therapy.

Cultures of all wounds were essentially the same, growing *Bacillus proteus, Bacillus aerogenes,* hemolytic *Staphylococcus aureus,* and

Clostridia. The aerobes were sensitive to 8, 2, and 1 units per ccm. of streptomycin, respectively. Local therapy was employed, resulting in prompt cessation of drainage from the right ear and from the sinus of the hip. The suprapubic wound also closed rapidly, only to reopen several days later, with release of pus under tension. Apparently, there was pooling deep in the space of Retzius, with sealing off by the healed overlying structures. The local therapy had failed to make contact with a deep focus of infection. He was then given three days of systemic therapy, and the wound promptly stopped draining and healed. There has been no recurrence of infection, and the wounds have remained healed.

Comment: This case, like the preceding one, emphasizes the importance of combined systemic and local therapy to clear infection from chronic, thick-walled, poorly-vascularized sinus tracts. Pus must be drained. Streptomycin will not cause it to disappear. The advantage of combined local and systemic therapy lies in the high concentrations of drug delivered to the site of the lesion.

Discussion

These illustrative cases represent serious infections of from four to eighteen months duration, in which the treatment, prior to streptomycin, had included prolonged chemotherapy, especially sulfonamides and penicillin, locally, systemically, and in combination, without success. They typify a group of chronic indolent infections in which dead tissue is always present, bone is usually involved, foreign bodies frequently are present, and complicated, burrowing sinus tracts into surrounding soft part structures are sequelae. Usually, there is a putrid, purulent wound exudate. Cultures yield a polymicrobic flora of mixed gram-positive and gram-negative organisms, both aerobic and anaerobic. It is frequently impossible to tell which are the contaminants and which are the true etiological agents. All are symbiotic infections due to several organisms. In the majority of instances, the gram-positive bacteria have become penicillin-fast.

In selecting patients from such a group for streptomycin therapy, it is imperative to employ the drug in conjunction with, or as soon as possible after, removal of foreign bodies and devitalized tissue. Pus must be drained. The drug must penetrate all reaches of the disease, in inhibitory concentrations which must be maintained long enough to eliminate infection. Otherwise, reinfection will occur as soon as the bacteriostatic effect of the drug is spent.

INFECTIONS OF THE URINARY TRACT

The efficacy of streptomycin in the treatment of infections in the genito-urinary tract, in 18 patients with neurogenic bladders due to spinal cord injuries, was evaluated. These patients are notoriously subject to a variety of urinary tract infections, including pyelonephritis, cystitis, urethritis, periurethral abscess, prostatitis, and epididymitis. These complications have been treated heretofore, with variable success, with other urinary tract antiseptics. Removal of suprapubic tubes with closure of cystostomy wounds, the discontinuance of repeated catheterization, together with the establishment of an automatic bladder, sometimes lead to spontaneous subsidence of the infection. Other infections may clear up following removal of calculi. In patients with upper urinary tract infections, bed rest, forcing of fluids, and elevation of the foot of the bed in order to promote peristalsis of the ureter and ballottement of the kidneys, are valuable adjuvants to specific treatment. However, a substantial number of infections are resistant to all these measures.

The organisms causing urinary tract infection in these patients are, in the order of frequency: *Streptococcus non-hemolyticus, Proteus vulgaris, Aerobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus hemolyticus.* In nearly all cases, more than one organism is present in the infected urine or pus. The non-hemolytic streptococcus, which heads the list of organisms, is insensitive to penicillin and to the sulfonamides. Most of the other organisms are gram-negative bacilli which, also, are resistant to penicillin and the sulfonamides. In contrast, all groups, both gram-positive and gram-negative, are inhibited by concentrations of streptomycin which readily can be achieved in the urine by parenteral administration.

We have treated 18 patients with varying doses of streptomycin. All but one patient had traumatic transverse myelitis with paraplegia and neurogenic bladder, and secondary urinary tract infection. The patient without the spinal cord injury was a 51 year old white male who had prostatic hypertrophy, and multiple diverticula of the bladder, with urinary retention and chronic cystitis. The infection persisted after diverticulectomy and prostatectomy, in conjunction with penicillin and the sulfonamides. The patient's symptoms consisted of marked urgency, burning on urination, frequency of urination averaging 10 times a day, and nocturia averaging about 8 times a night. Culture of the urine showed *Bacillus proteus, Aerobacter aerogenes,* and *Streptococcus non-hemolyticus,* which were sensitive to 64 units, 8 units, and 16 units of streptomycin per ccm., respectively. This patient received 200,000 units of streptomycin, intramuscularly, every four hours for 18 doses, the total dosage being 3,600,000 units. After the first day of treatment, the nocturia was reduced to 3 times a night, and the urgency and burning were definitely decreased. By the third day of treatment, the patient was completely asymptomatic. At the conclusion of treatment, urine culture revealed only *Streptococcus non-hemolyticus.* Two weeks after the conclusion of treatment, the urine was sterile. This patient was still asymptomatic two months later.

TABLE 5

ANALYSIS OF BACTERIAL FLORA IN URINE OF PATIENTS BEFORE AND AFTER TREATMENT WITH STREPTOMYCIN

Organism	Number of organisms before treatment	Number at conclusion of treatment	Number 2 weeks after treatment
A. *Cases* 1 to 7 inclusive, treated with 200,000 units of streptomycin every 4 hours for 3 days.			
Strep. non-hemolyticus	7	6	5
B. proteus	7	5	6
A. aerogenes	4	0	2
E. coli	3	1	1
Ps. aeruginosa	2	0	0
K. pneumoniae	1	0	0
Staph. aureus	1	1	0
B. *Cases* 8 to 13 inclusive, treated with 150,000 units of streptomycin every 3 hours for 3 days. (Case 13 received 200,000 units every 3 hours for 3 days.)			
Strep. non-hemolyticus	6	6	6
B. proteus	6	4	5
A. aerogenes	3	0	1
K. pneumoniae	3	1	3
Ps. fluorescens	1	0	1
E. coli	1	1	1
C. *Cases* 14 to 18 inclusive, treated with 375,000 units of streptomycin every 3 hours for 3 days. (Case 18 received 400,000 units every 3 hours for 3 days.)			
Strep. non-hemolyticus	4	2	3
B. proteus	4	0	3
A. aerogenes	3	0	3
K. pneumoniae	2	0	0
E. coli	1	0	1
Staph. aureus	1	0	0
Strep. hemolyticus	1	0	0

Comment: This patient was the only one in this series of urinary tract infections who did not have a neurogenic bladder. He had no urinary obstruction, at the time of treatment, no calculi, and required no catheterization. The favorable result obtained with streptomycin must be considered in the light of these circumstances, when compared with the other cases in this group.

Twelve patients with spinal cord injuries were treated, for 3 days, with streptomycin doses of either 200,000 units every four hours or 150,000 units every three hours. In contrast to the above case, in only one instance was the urine sterilized, and even then reinfection occurred within two weeks. In the other cases, some of the bacteria disappeared during treatment, only to reappear after treatment. These patients had, variously, one or more of the following complications which, we feel, contributed to the failure of streptomycin therapy to achieve complete and lasting sterilization: suprapubic cystostomies, urinary calculi, indwelling catheters, or residual urine requiring catheterization.

TABLE 6

CHANGE IN SENSITIVITY OF ORGANISMS TO STREPTOMYCIN FOLLOWING TREATMENT

Case	Organism	*In vitro* sensitivity (Units per cc.)		Total dosage of drug administered
		Prior to treatment	Two weeks after treatment	
3	*B. proteus* *A. aerogenes*	4 32	64 32	3,600,000 units
4	*B. proteus* *Strep. non-hem.*	16 16	16 16	3,600,000 units
5	*A. aerogenes* *B. proteus* *Strep. non-hem.*	128 16 16	over 128 16 16	3,600,000 units
6	*B. proteus*	16	over 128	3,600,000 units
7	*B. proteus* *Strep. non-hem.*	8 16	over 128 over 128	3,600,000 units
9	*B. proteus* *A. aerogenes*	16 16	8 16	3,600,000 units
10	*B. proteus* *Strep. non-hem.*	2 8	8 less than 0.5	3,600,000 units
11	*B. proteus* *Ps. fluorescens* *Strep. non-hem.*	4 8 4	over 256 over 256 8	3,600,000 units
12	*B. proteus* *Strep. non-hem.*	4 4	over 256 over 256	3,600,000 units

On the basis of this experience, the dosage of streptomycin was increased to 375,000 units every three hours for 24 doses, a total of 9,000,000 units. In five patients treated with this dosage, the urine was sterilized in all, except that the non-hemolytic streptococcus persisted in two cases. TABLE 5 illustrates vividly the striking effect on the bacterial flora of the larger doses, as compared with the smaller doses. However, it is to be noted that reinfection with some bacteria occurred in all cases, regardless of dosage, emphasizing the role of calculi, open wounds, and residual urine, in the failure of streptomycin to achieve complete and permanent sterilization of the urinary tract.

Bacteria in the urinary tract rapidly acquire resistance to streptomycin (TABLE 6). Inadequate treatment, whether due to insufficient dosage, or presence of the other causes for reinfection already enumerated, may lead to the rapid development of drug-fastness. We, therefore, feel that streptomycin is contra-indicated in the presence of suprapubic fistulae, urinary calculi, residual urine, mechanical obstruction, or any condition requiring repeated catheterization. The drug should be reserved for such time as these conditions are corrected. Interestingly enough, the development of drug resistance was not found as consistently in our group of infections in battle wounds, except with *Pseudomonas aeruginosa*.

BACTERIEMIAS

The third group of cases which we studied consisted of four patients with bacteriemia.

Case 1: This patient was a 22 year old paraplegic who, on 20 June, 1945, suddenly developed an acute illness manifested by repeated severe shaking chills and a "spiking" temperature which reached 106.8°. On the fourth day of the illness, blood culture was reported positive for an organism in the Friedlander-Aerogenes group. Streptomycin therapy was begun with an intravenous dose of 1,000,000 units, dissolved in 300 ccm. of 5% glucose in normal saline. In addition, the patient received 250,000 units, intramuscularly, every 90 minutes, the total daily intramuscular dosage amounting to 4,000,000 units. The temperature subsided by lysis, becoming normal on the sixth day of treatment. On the eighth day of treatment, the temperature rose to 101.6°, and patient developed a generalized morbilliform rash. Streptomycin was then discontinued, with the result that the temperature subsided promptly, and the rash disappeared. Daily blood cultures taken during the illness were all negative subsequent to the institution of streptomycin therapy.

Comment: The bacteriemia was controlled by the large dosage of streptomycin employed. The secondary rise in temperature which occurred on the eighth day, and the rash, may be considered as toxic reactions caused by the drug.

Case 2: This was a 24 year old paraplegic, who developed repeated shaking chills and fever on 1 July, 1945, the temperature rising to 105°. Two blood cultures, taken during different chills on 3 July, 1945, were reported positive for an organism in the Friedlander-Aerogenes group. Streptomycin therapy was begun on 4 July, 1945, with intramuscular doses of 200,000 units every four hours, amounting to 1,200,000 units daily. Although blood cultures became negative, the chills and fever persisted unchanged for 3 days. The dose was then increased to 300,000 units every 3 hours, the total daily dose amounting to 2,400,000 units. This was followed by a slow drop in the temperature to 101°, but the patient continued to have several mild chills daily. On 13 July, 1945, streptomycin was discontinued. The temperature promptly increased, and the chills became more severe. On 20 July, 1945, an intravenous pyelogram revealed the presence of a calculus in the right ureter. On the next day, ureteral catheterization was done, and about 100 ccm. of thick foul creamy pus was aspirated from the right ureter. Culture of this pus revealed *Bacillus proteus*. A blood culture taken on the previous day grew the same species, but no Aerogenes-Friedlander organism.

On 21 July, 1945, streptomycin therapy was resumed on a dosage schedule of 375,000 units every three hours, the daily dosage amounting to 3,000,000 units. Ureterolithotomy was performed, on 24 July, 1945, following which the temperature subsided, and the patient made a clinical recovery. Streptomycin was discontinued on 27 July, 1945, the patient having received a total of 37,350,000 units.

At the onset of the illness, the following bacteria were cultured from the urine: *Klebsiella pneumoniae, Bacillus proteus, Escherichia coli,* and *Streptococcus non-hemolyticus,* each sensitive to 16 units of streptomycin per cc. *K. pneumoniae* and *E. coli* disappeared within one day after institution of specific therapy, but *B. proteus* and *Streptococcus non-hemolyticus* persisted throughout and after treatment. Tests after the conclusion of treatment showed that the growth of *B. proteus* was not inhibited *in vitro* by 50,000 units of streptomycin per cc. In other words, the resistance of *B. proteus* to streptomycin, in this case, had increased from 16 units per ccm. to over 50,000 units per ccm.

Comment: The relative ineffectiveness of streptomycin, in this case, contrasts sharply with its effectiveness in the former case. The lack of response may be attributed partly to small early dosage, but chiefly to the failure to evacuate pus from the ureter and to remove the calculus obstructing drainage, until late in the course of the disease. The marked increase of resistance to streptomycin of *B. proteus,* because of inadequate treatment, is noteworthy.

BRUCELLOSIS

Case 1: A 30 year old bacteriologist was admitted to the hospital, 27 July, 1945, complaining of "high fever" and "weakness" of two days duration, preceded by a mild diarrhea. The fever persisted and blood cultures were positive on 3 different occasions, for *Brucella melitensis,* which was determined to be sensitive *in vitro* to 1 unit of streptomycin per ccm. Streptomycin therapy was instituted, 11 August, 1945, the patient receiving 200,000 units, intramuscularly, every four hours for 10 days. The fever subsided by lysis, becoming normal on the eighth day of treatment. On 22 August, 1945, a thrombophlebitis of the deep veins of the left leg developed, but subsided gradually. On 8 September, 1945, the patient developed pneumonia, which responded promptly to penicillin.

Two other febrile episodes, associated with malaise and generalized lymphadenopathy, occurred during September, 1945. In October, 1945, another such episode occurred, during which blood cultures again were positive for *Brucella melitensis.* Streptomycin therapy was resumed, the patient receiving 333,000 units every four hours for 10 days. The febrile course was unchanged, the temperature fluctuating daily up to 102° F. However, blood cultures became negative. The streptomycin level in the blood was 16 units per ccm. The temperature returned to normal, one day after cessation of treatment, but rose again, after a short interval.

Comment: Streptomycin was undoubtedly a failure in this case, which showed the typical exacerbations and remissions to be expected in undulant fever.

TYPHOID FEVER

Case 1: A 24 year old soldier developed classical signs and symptoms of typhoid fever, confirmed by positive blood and stool cultures. Streptomycin therapy was instituted in the third week of the illness, the patient receiving 500,000 units intramuscularly and 160,000 units orally, every four hours for six days. Stool and blood cultures were still positive, 24 hours after institution of therapy, but were negative thereafter. Although the patient's clinical appearance seemed to improve, the temperature persisted throughout the treatment, and subsided later by lysis.

Comment: Although the cultures became negative, and the clinical appearance improved, the temperature persisted, as in the usual course of this disease. The role of streptomycin in the patient's recovery is difficult to evaluate.

DRUG REACTIONS

We have noted no serious reactions to streptomycin therapy. In several cases, generalized morbilliform eruptions have occurred (PLATE 8), with or without a febrile reaction, eosinophilia, and albuminuria. These cleared up promptly, on discontinuance of the drug. In many cases, a reducing reaction has been observed in the urine. This reaction is due, chiefly, to the presence of streptomycin itself in the urine (PLATE 9). Some batches of the drug cause a transient drop in systolic and diastolic blood pressure, without accompanying symptoms. We do not feel that these reactions contraindicate further drug administration, if the patient's condition justifies it.

CONCLUSIONS

1. Streptomycin inhibits the growth of gram-negative and gram-positive pathogenic bacteria. The Colon and Friedlander's group are especially sensitive, while the Proteus, Aerogenes, and Pyocyaneus groups are more resistant to this drug.

2. Non-hemolytic streptococci and hemolytic staphylococci require 4 to 8 times more streptomycin to inhibit their growth when human blood, plasma, or serum is added to the media.

3. Though there is no correlation of the sensitivity of organisms to penicillin and streptomycin, subinhibitive doses of each, when combined, have an additive effect.

4. To combat aerobic, pathogenic bacteria of surgical infections, a sustained minimum blood concentration should be not less than 8 units per ccm. To accomplish this, 400,000 units of streptomycin should be given intramuscularly every four hours. In surgical infections, treatment should be continued for 7 days or longer; in genito-urinary infections, for 3 days or longer.

5. The importance of adequate surgery is emphasized, if streptomycin therapy is to succeed in eliminating infection when dead tissue, collections of pus, foreign bodies, calculi, and thick-walled sinus tracts are present.

6. Too small a dosage, too short a course of treatment, or failure to evacuate pus, calculi, sequestrae, or foreign bodies, predispose to the prompt development of drug-fastness.

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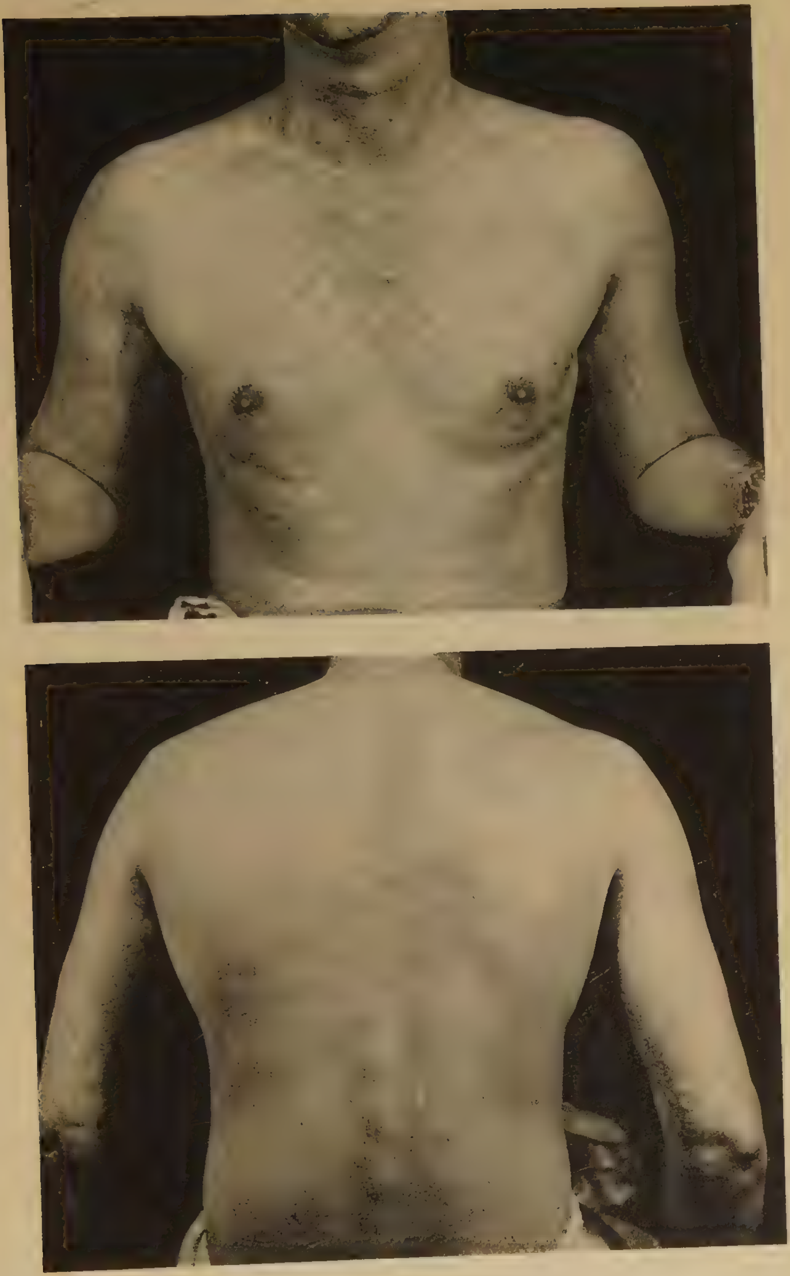
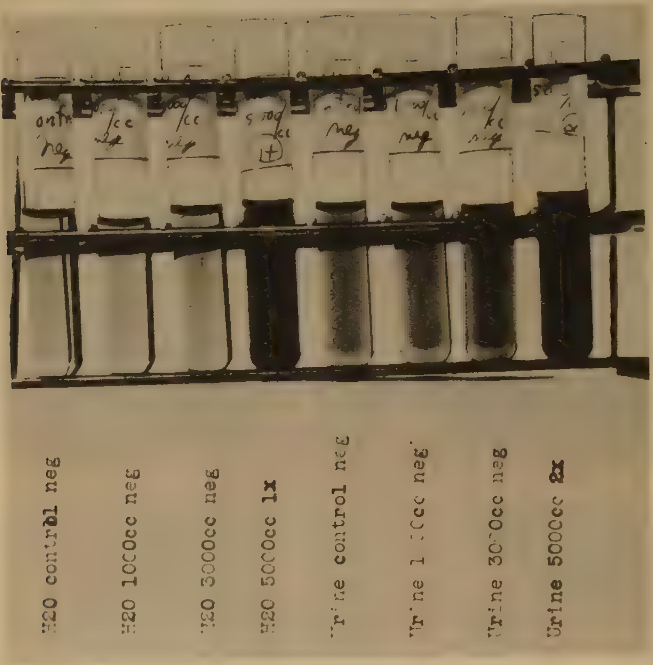
PLATE 8

Generalized morbilliform eruption in reaction to streptomycin therapy; 8th day of administration; 0.4 gm, every four hours, intravenously.



PULASKI AND OTHERS: CLINICAL USES OF STREPTOMYCIN



PULASKI AND OTHERS: CLINICAL USES OF STREPTOMYCIN

PLATE 9

Reducing effect of streptomycin on alkaline copper solutions.

CLINICAL EXPERIENCES WITH PENICILLIN IN THE NAVY[*][†]

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In March, 1945, the Penicillin Committee of the National Naval Medical Center[1] published a comprehensive report on the treatment with penicillin of 1,455 cases, for fifty different clinical entities. The unrestricted availability of penicillin, since that time, has encouraged medical officers to utilize this chemotherapeutic agent in a variety of conditions. This report, including the figures from the National Naval Medical Center, has raised the number of reported conditions under treatment with penicillin to sixty-five, and the aggregate of patients treated, to 17,879. In order to make this a comprehensive picture of the use of penicillin, in the Navy, not only my own experiences in the treatment of syphilis, gonorrhea, and dermatologic conditions have been utilized, but the published reports of Naval Medical Officers the world over have been freely drawn upon, and some unpublished data from colleagues in special fields have been included.

The history of the discovery of penicillin and its later development as a practical chemotherapeutic agent is now well known to the medical profession and will not be reviewed. Likewise, it is beyond the scope of this paper to delve into the chemistry or pharmacology of the drug. It shall, therefore, only summarize its clinical application, as it has been used in the Navy.

CLINICAL APPLICATION OF PENICILLIN

Penicillin in Pneumonia, Lung Abscess, Bronchiectasis, Empyema, Acute Pleurisy, and Pleural Effusion[1-11]

In this group, a total of 1,694 cases, including two cases of influenza bacillus pneumonia, was treated with penicillin alone, or in combination with sulfonamides and supplementary procedures. In primary, atypical pneumonia, penicillin was of definite value in combating secondary bacterial invaders, reducing the febrile period, and lowering the number of sick days. In lobar and bronchopneumonia, using a minimal dose ranging from 409,000 to 2,084,000 units, depending on the type and complication present, success was more consistent when penicillin was used immediately, rather than after the delay incurred by the prior use of a sulfonamide. In the latter instance, larger doses were needed and more failures encountered. A marked reduction in the development of complications was noted, when the initial dose consisted of 200,000 units, intravenously given in 200 ccm. of 5% glucose and saline, followed by 100,000 units intravenously every two hours. A slow clinical response was the indication for giving an additional intravenous dose of 200,000 units, every four to six hours, as a booster. Excellent results with this time-dosage relationship were achieved in two cases of influenza bacillus pneumonia, an organism which, heretofore, has not been considered susceptible to penicillin. This method of administration, together with the limitation of fluids, produced high blood levels, and obviated pulmonary edema. An appreciable number of complicating empyemas escaped surgery, after intrapleural instillations of penicillin. Four pneumonias, complicated by rheumatic fever, responded, without improvement in the status of the rheumatic fever.

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[*] A report based on 17,879 cases treated for sixty-five clinical entities.

[†] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth in this article are those of the writer and are not to be construed as reflecting the policies of the Navy Department.

Although chemotherapeutic measures have not altered the fundamental principles for surgical drainage, in frank empyema or lung abscess, the early and effective use of the former have, to a degree, eliminated the need for the latter. In a group of 37 cases of streptococcal empyema, 29 responded to aspiration, with the instillation of 20,000 units of penicillin intrapleurally, after each aspiration, and an additional dose of 5,000 units I.M., every three hours. The average number of hospital days, for the aspirated cases, was 84.5 days. In contrast to this, the requirements of the eight patients who were treated surgically were in excess of 167 days.

Of four cases of lung abscess, two responded to penicillin alone, while two required surgical drainage, in addition. In all instances, the toxemia was reduced, and the course of the disease shortened.

One case of acute fibrinous pleurisy, in which the cause was not determined, responded promptly to penicillin within 48 hours. Excellent results were obtained, in a case of severe unilobular bronchiectasis, by daily bronchial lavage and intrabronchial instillation of penicillin or sulfonamide solutions, after more conventional methods of treatment had failed. The use of this method is suggested for those cases in which surgery is not advisable or desired.

From the accumulated evidence at hand, one fact can be emphasized: that, in pneumonia, the mortality and complications are reduced if penicillin is used early and in preference to the sulfonamides. Not only is it the drug of choice, in pneumococcal, staphylococcal, and streptococcal pneumonia, but evidence is also accumulating that its use is justified in primary, atypical pneumonia.

Meningitis and Meningococcemia[1, 12-14]

This study comprises fifty-four cases, in which the meningococcus was the offending organism in forty-six; the pneumococcus, in six; and the streptococcus, in two. The unquestioned value of penicillin, for the treatment of meningococcal infections, has been well established, particularly since it offers the added advantage of bringing about a satisfactory response, when the sulfonamides fail, and supplants the latter, in the treatment of patients in whom sulfonamide therapy is contraindicated. All of the meningococcus-meningitis cases responded to the systemic administration of relatively minimal doses of penicillin (total of 40,000 units, over a period of 8 hours, to a maximum of 250,000 units, over a 48 hour period), with an additional injection or two, intrathecally, of 10,000 units.

Regardless of whether penicillin or sulfadiazine is used in the treatment of meningococcic infections, it is imperative that prompt, adequate blood levels be established and maintained, because, when death occurs during the first three or four days, it is from the septicemia rather than the meningitis. In order to conserve valuable time, and in the interest of saving lives, it has been the established practice, at the U. S. Naval Hospital, San Diego, to institute concurrent sulfadiazine and penicillin therapy, rather than to exclude one or the other, or postpone treatment until bacteriologic study involving the penicillin sensitivity of the isolated organism has been completed. These methods are time-consuming, and more expensive than the small amount of penicillin necessary to determine whether it will be of value in the particular case. Response is usually prompt, when the drug is of any value, and can be discontinued when the bacteriologic and clinical signs of improvement become well established.

It has, also, been found that penicillin, administered systemically, can diffuse into spinal fluid in adequate concentration during the course of severe meningitis. For this reason, intrathecal therapy has been practically discontinued for almost a year. However, especially when early treatment has been neglected, failure of the patient to improve after accepted forms of treatment, coupled with failure of establishment of adequate blood levels, should always suggest the possibility of spinal block. In this event, the more direct approach through the cisterna magna, with the administration of penicillin, cisternally, after drainage of fibrinopurulent material, has been found to be of value and devoid of danger. Forcing of fluid, beyond maintaining a urinary output of 1500 ccm. per day, is not necessary. In fact, it is believed that restriction of fluids is of definite value, in production of the bilateral effect of maintaining higher blood levels of the drug, and forestalling pulmonary edema, which was, without exception, a postmortem finding.

The cases of meningococcemia deserve special consideration, because they showed a mortality of 70 per cent. Of the survivors, one case of meningococcemia was complicated by gangrene of fingers and toes, which led to residual deformities of the terminal phalanges of three fingers. Within twelve to forty-eight hours of penicillin therapy, definite improvement, with recovery of consciousness, decreased rigidity of neck, and sharp decline in fever, was noted. Intramuscular penicillin was used, from 5 to 10 days, in a dosage of 30,000 units, every 2 or 3 hours; and intrathecal penicillin, for 3 or 4 days, in a dosage of 10,000 units in concentration of 1000 units per ccm., every twelve hours.

In a case of meningococcemia with arthritis, penicillin therapy was instituted, 14 days after intensive sulfadiazine therapy failed to control a persistent meningococcemia with acute arthritis of both knees. After 10 days of penicillin, intramuscularly, in dosage of 15,000 units every 3 hours, the fever declined by lysis, and the arthritis subsided, after initial aspiration. The blood culture remained positive for a type I meningococcus, for the first seven days, and recovery was complete, although arthritic manifestations persisted for an additional 6 weeks.

The other case of fulminating meningococcemia with purpura (Waterhouse-Friderichsen syndrome) survived, with massive doses of penicillin (46 million units total), restriction of fluid intake to 2500 ccm. daily, and sulfadiazine. This case was complicated by gangrene of nine toes, which were later amputated.

Out of this entire group of 54 cases, there were 9 deaths, distributed as follows: Seven cases of meningococcemia, one of pneumococcic meningitis in a 58-year old man, and one case of meningococcus meningitis who expired 38 hours after admission, and in whom necropsy disclosed suppurative meningitis, secondary hydrocephalus, and edema of the brain and lungs. (He had received 300 ccm. of additional fluids, daily.)

Scarlet Fever, Erysipelas, Cellulitis, and Stomatitis[1, 15–19]

This group includes a comparative study of two thousand cases of scarlet fever which were hospitalized, from 1943 to 1945. From 1943 to 1944, the sulfonamides were employed routinely, and penicillin deferred for 48 hours, when it was started because of failure of response to the sulfonamide or the development of a complication jeopardizing the patient's life or prompt recovery. During this year, there were seven deaths. In the thousand cases observed, during 1944 to 1945, penicillin was not deferred, but given early in a dosage of 25–50,000 units intramuscularly, every two to three hours. Critically ill patients received 100,000 units intramuscularly, every hour or two, until the crisis was passed. There was one death, in this latter series.

The cases of cellulitis included infection of the jaw and neck, following extraction of a tooth; diffuse, deep maxillary cellulitis, secondary to infection of the submaxillary gland, following blockage of the duct by three calculi; and two cases of infection of the hand, following human bite. Bacteriological studies, in these cases, revealed mixed infections with the streptococcus, non-hemolytic *Staphylococcus aureus*, diphtheroids, and beta-hemolytic streptococcus. All responded to penicillin therapy, without surgical interference, including the deep submaxillary cellulitis, in which surgery is usually advocated. The total dosage varied, from 720,000 units to the 2,050,000 units which were administered in a case of Ludwig's angina. The bite infections responded to a total dosage of 1 million units, given by the intramuscular route, and were completely healed in ten days. In this connection, penicillin-blood plasma powder, applied directly to infected wounds, has been observed to be highly effective in controlling purulent discharge and promoting healthy granulation tissue. It combines bacteriostasis with excellent nutrients for tissue regeneration.

Penicillin, when administered for erysipelas, stomatitis, and Vincent's angina, gave uniformly good results. In forty-one cases of the latter, twenty-nine were cured with a total dose of 200,000 units, given at the rate of 10,000 units intramuscularly, every three hours. The remainder cleared, after a second course given the following day. The average number of hospital days was decreased to five, in these cases, as compared with twenty-three, for those treated by all other methods (TABLE 1).

Otorhinolaryngology[1, 20, 21]

In the special field of diseases of the ear, nose, and throat, particularly in otitis media and its complications—sinusitis, pharyngitis, tonsillitis, and suppurative pharyngeal lesions—penicillin, both systemically and topically, is by far the drug of choice. Most often, these conditions are caused by penicillin-sensitive organisms, and the early and adequate use of penicillin has appreciably reduced the incidence of serious and often fatal complications. As a rule, acute otitis media responded, in 2 to 4 days, with continuous intramuscular injection of 200,000 units per day, some becoming afebrile in 24 hours. In those complicated by mastoiditis necessitating surgical drainage, the prior use of penicillin permitted of a simple mastoidectomy, and the continued systemic use of penicillin after surgery, together with the instillation of penicillin solution into the wound, brought about prompt healing of the middle ear and complete healing of the wound, in 8 days.

Table 1*

Clinical Data in 41 Penicillin-Treated Cases of Vincent's Angina

	Areas Involved			
	Tonsils	Gums	Gums and tonsils	Totals
Number of cases involving	25	13	3	41
Cases cured by 200,000 units	15	11	3	29
Cases cured by 400,000 units	10	1	1	12
Average hospital days after start of penicillin	6	4.5	4.5	5.4
History of previous attacks, failure of other treatments	13	4	1	18

* Reprinted from **Twining, H. E.,** *et al.* U. S. Naval Med. Bull. **45:** 481. 1945.

Maxillary sinusitis responded rapidly to the topical use of penicillin following irrigation, and, not infrequently, this form of therapy changed a frankly purulent to a mucoid discharge, in 24 hours, with prompt cessation of local discomfort. In the more severe infections, the additional systemic administration of penicillin is advocated. In one case of acute ethmoiditis, complicated by orbital cellulitis and septicemia, the response to penicillin, given intramuscularly at the rate of 20,000 units every 3 hours, was dramatic, after a total dose of sulfadiazine of 310 grams and two blood transfusions in two days failed to improve the clinical course of the disease. The temperature dropped from 105° F. to 102.6° F., in 24 hours, and free drainage from the nostril was established. Five days after penicillin therapy was started, the orbital abscess was incised and drained, followed by complete recovery.

The *Staphylococcus aureus* was recovered from the nasal drainage, the blood stream, and the orbital abscess.

Because of a gradual rise of complicating mastoiditis, in a large series of cases of otitis media, and the apparent failure of sulfonamide therapy, a program of penicillin therapy was instituted, with striking and gratifying results (TABLE 2). The incidence of mastoiditis dropped from 27.6 per cent, in one month, and only four patients who had received adequate penicillin therapy developed surgical mastoiditis. Adequate treatment consisted of continuous treatment for four days, prior to the onset of signs and symptoms of mastoiditis.

Table 2

Incidence of Surgical Mastoiditis and Complications of Otitis Media

(From **von Christierson, S.**[20] U. S. Naval Med. Bull. **45**: 920. 1945.)

Month	Otitis media	Mastoidectomies	Per cent	Complications
August 1943	51	2	4.0	0
September	91	7	7.7	2
October	64	1	1.7	0
November	58	5	8.6	1
December	123	17	13.8	0
January 1944	113	30	26.5	7
February	120	20	16.6	5
March	186	23	12.4	6
April	123	*34	27.6	5
May	113	7	6.2	2
June	87	6	6.8	0
July	90	6	6.6	1
Totals	1,219	157	12.9	29

* One patient who had mastoiditis with cerebellar abscess and petrositis was not operated upon.

Penicillin in Ophthalmology[1, 22]

In the field of ophthalmology, penicillin has both a local and systemic use. Mild conjunctivitis, blepharitis, and superficial ulcer responded to the instillation of penicillin in a concentration of 500 units per ccm., supplemented by penicillin ointment. Systemic therapy, however, is indicated in gonorrheal ophthalmia, orbital cellulitis, and intraocular infections. In one case of severe iridocyclitis, penicillin was used, with complete recovery, after atropinization, continuous hot compresses, topical applications of sulfanilamide powder to the conjunctival sac, and intravenous typhoid therapy had been used, without improvement in the condition. After two days of intramuscular penicillin, at the rate of 20,000 units every three hours, the ciliary injection was definitely lessened, and the pupil was well dilated. The patient was discharged as cured, 2 weeks after admission.

Appendicitis and Peritonitis[1, 23]

Penicillin, in a dosage of 20,000 units every two hours for 5–7 days, was found effective in controlling peritonitis associated with ruptured appendix, in seven cases. In general, however, the smaller dosages will not prevent the formation of intraperitoneal abscesses. Twenty-five patients, with extensive contamination of the peritoneal cavity, were treated with doses of 100,000 units given intramuscularly every 2 hours for 2 days; then, 50,000 units, every 2 hours for 2 days; followed by 50,000 units, every four hours for 2 days; and finally, 25,000 units, every four hours for 2 days; a total dosage of 4,500,000 units, in 8 days. The treatment was followed by prompt recovery, without intra-abdominal complications, and with no residual abscesses. The high initial dose was felt advisable, in order to overcome the penicillin-neutralizing effect of the *Escherichia coli.*

In two cases of intestinal obstruction with peritonitis, following rupture of the small intestine, penicillin was unsuccessful, in one, and its action was indeterminate, in the other. In both cases, the intestinal flora was predominantly gram-negative.

Brain Abscess[24]

Penicillin has been found to be a valuable adjunct to surgery, but is not, in any sense, a substitute for surgery, once a brain abscess becomes established. In six cases in which the sulfonamides had been used in adequate dosage, for a period of five to seven days, without abatement of symptoms, the introduction of penicillin brought about prompt improvement, and five survived, after surgical drainage (TABLE 3). Bacteriologic studies revealed the hemolytic streptococcus, hemolytic *Staphylococcus aureus,* and a non-hemolytic streptococcus, as the offending organisms.

Penicillin in the Treatment of Yaws[25, 26]

During the past year, the Medical Department of the Navy has been conducting a study of the efficacy of penicillin in the treatment of yaws, among the natives of Samoa.

Of the cases treated to date, 60 were of early yaws, 30 of late yaws, and 19 were unclassified. Cases classified as early received 20,000 units of penicillin intramuscularly, every 3 hours for 8 days, and the late cases received the same dosage, for 21 days. The total doses were, therefore, 1,280,000 units and 3,600,000 units, respectively. The unclassified cases were treated during a preliminary phase of the in-

TABLE 3

PENICILLIN AS AN ADJUNCT TO SURGERY IN BRAIN ABSCESS

Reprinted from **Furlow, L. T.** Penicillin as an adjunct to surgery in the treatment of brain abscess. Southern Med. J. 38: 317. 1945.

Name and case No.	Age	Etiology	Neurological symptoms	Location	Culture	Sulfa. therapy	Results	Penicillin therapy	Results	Final result	Remarks
B.P.W. *Case 1* (M)	24	Scarlet fever, frontal sinusitis	Headache, mental confusion, reflex changes on theright, aphasia, papilledema	Left frontal lobe. Left parietooccipital lobe not drained	Hemolytic streptococcus	Yes, adequate	Progressive decline during therapy	900,000 units, two series	Temporary improvement in general condition	Death	Second abscess not drained
F.B. *Case 2* (M)	3½	Compound depressed skull fracture, right frontal bone	Meningeal signs, fever, coma	Rt. frontal lobe	Hemolytic *Staph. aureus* Diphtheroid bacilli	No		800,000 first series. 1,100,000, second	Localization and subsidence of infection	Cured	
C.M.R. *Case 3* (M)	19	Pneumonia, pleurisy	Headache, vomiting, papilledema, bilateral, sixth nerve paresis	Left posterior parietal. Two abscesses	Hemolytic *Staph. aureus*	Yes, intravenously to level of 13 mg. per cent	No improvement	235,000 units intravenously. 500,000 units intramuscularly, first. 700,000, second	Improvement	Cured	
C.W.P. *Case 4* (M)	20	Otitis media, mastoiditis	Convulsions, reflex changes on right	Left temporal lobe	Negative (smear-staph.)	Yes, when penicillin supply exhausted	No improvement	880,000 units, intramuscularly in two series	Improvement	Cured	
R.G.R. *Case 5* (M)	18	Otitis media, mastoiditis	Meningeal signs, aphasia, rt. facial, reflex, changes, rt., variable stupor, quadrantic rt. hemianopsia	Left temporal lobe	Non-hemolytic streptococcus	Yes, adequate	No improvement	1,500,000 units, intramuscularly	Improvement	Cured	
B.V.H. *Case 6* (M)	36	Otitis media, mastoiditis	Meningeal signs, aphasia, reflex changes on right	Left temporal lobe	Non-hemolytic streptococcus	Yes, adequate	No improvement	1,200,000 units intramuscularly, first series, 100,000 intravenously. 920,000 intramuscularly, second series	Improvement	Cured	

vestigation and received total doses ranging from 200,000 to 3,200,000 units.

The early cases, with generalized, fresh, granulomatous lesions, were cured, clinically, more rapidly than were the later cases, with destructive types of lesions. Patients with generalized, fungating yaws, whose lesions were teeming with spirochetes, were cured, clinically, in 5 to 7 days. The late lesions, some of which had been present for long periods of time, healed at about the same rate as would any clean surgical wound. Pinch skin grafts, which took readily, were applied to some of the more extensive ulcers, in order to shorten the time of convalescence. Roentgenographic studies, in one case of yaws involving bone, showed that the destructive process had been checked by penicillin, and that there was an excess of dense, osseous tissue, at the site of the old lesion.

Darkfield studies demonstrated that the spirochetes disappeared, from the early lesions, within an average of 14 hours, and from the late lesions, in 13 hours. Darkfield examinations were negative, in 3 late cases of long standing which had been treated with neoarsphenamine and bismuth, without clinical improvement. These cases healed under penicillin therapy.

Four of the unclassified cases, treated during preliminary studies, are of unusual interest. Two of these patients were apparently cured with total doses of only 200,000 units, administered intramuscularly in doses of 5,000 units, at intervals of 3 hours. In one of these, the Kahn test was essentially negative, one year following treatment. A third case showed the usual prompt bacteriological and clinical response to penicillin administered over a period of 5 days (total dose 400,000 units). The Kahn test, which had been strongly positive, was nearly negative after six months. One year later, the patient developed a typical, raised granuloma of early yaws, in which spirochetes were found. The Kahn test, again, became strongly positive. It was concluded that this child had again acquired yaws, after having been cured of the original infection. A fourth patient, in the unclassified group, was the only therapeutic failure in the entire series of 109 cases. Although the darkfield examination was negative, after 24 hours, and the lesions healed, the Kahn test remained strongly positive. Four months later, there was a recurrence of foul, punched-out ulcers over the upper extremities and back, but these lesions showed no treponemata, on darkfield examination. The reason for the failure of penicillin and for the persistency of unusually destructive and extensive lesions, in this case, is not clear. (The etiology of these lesions was not completely established.)

Quantitative Kahn tests, performed at intervals of 3 weeks, showed, in general, a very slow reduction in titer and considerable fluctuation. The only exception was in the single therapeutic failure, previously described. The shortest time required for the Kahn test to become negative, in this series, was 5 months. In view of the similarity of *Treponema pallidum* and *T. pertenue,* the marked difference in rate of serological response is puzzling. Quantitative testing of sera from these patients will be continued, at appropriate intervals.

In summary, it may be stated that, during a relatively short period of observation, penicillin has proved highly effective in the treatment of yaws. Clinical response has been prompt, while serological response has been very slow and irregular. Final evaluation cannot be made until these patients have been followed over a longer period of time.

The method of administering penicillin used in this study required hospitalization. If it were adequate to give one daily dose of the drug by employing absorption-delaying methods, or if oral administration should prove effective, it might be possible to treat natives with penicillin on an out-patient basis. Mass treatment of natives, in the past, has been largely an administrative problem, which required weekly injections of arsenical or bismuth compounds and careful follow-up observations, with treatment at definite intervals, for a period of years. Whether or not mass therapy with penicillin can be made practical, remains to be seen.

A case of generalized yaws, in a white man, responded to penicillin given intramuscularly, at the rate of 15,000 units every 3 hours, for a total dose of 1,500,000 units. Spirochetes could not be demonstrated in the lesions, 18 hours after treatment was begun; all lesions, except the ulcer, were healed in 5 days; and the ulcer itself, in 13 days. The blood Kahn reaction was positive, before the treatment was begun, and was still positive, when the patient was discharged from the hospital after 5 weeks of treatment, becoming negative one week later.

A total of 110 cases has now been reported, all showing prompt response, but final evaluation cannot be made, until these patients have been under observation for a longer period of time.

Diphtheria[27]

A study of 30 cases of diphtheria and diphtheria carriers revealed that penicillin does have a bacteriostatic effect on the *Corynebacterium* *diphtheriae.* The daily dosage of penicillin from which the best result was obtained was 100,000 units. Four patients responded to the 100,000-unit daily dosage, and one patient failed to respond. Of 20 patients treated with a 50,000-unit daily dosage, 12 (60%) had negative throat cultures, and 8 (40%) did not respond. Six of the patients who failed to respond to 50,000 units of penicillin daily did respond to a second course, consisting of 100,000 units daily. Three patients, treated with 25,000 units of penicillin daily, had throat cultures persistently positive for *Corynebacterium diphtheriae;* two of these responded to a second course, consisting of 100,000 units.

Gonococcus Urethritis[1, 28–34]

In this series of cases, 11,328 male patients, with gonococcus infection of the urethra, were treated, more than 50% of whom were classified as sulfa-resistant. A number of different schedules of dosage were followed, as well as routes of administration. Initially, the drug was given by continuous intravenous drip. Then, intramuscular injection, with or without local instillation of dilute penicillin solution into the urethra, was employed. No advantage was found in the local administration; hence, this method was discarded. The schedule found effective was 20,000 units given intramuscularly, every 3 hours, for five doses. This total dose of 100,000 units resulted in cure in 98% of the cases. Furthermore, in the 2% which were recorded as initial failures, cure was obtained by administering an additional 100,000 or 200,000 units. No case was found in which the gonococcus was completely resistant to penicillin. Although, in our series, the rate of relapse, after initial treatment, was a trifle higher, all responded on retreatment, which, in some cases, included the use of sulfadiazine (TABLE 4). Recently, more effective results have been obtained with a dosage of 25,000 units given intramuscularly, every 3 hours, for 6 doses. The early detection of penicillin-resistant strains, in any case, and the prompt modification of therapy to meet individual needs, have possibilities of reducing to nil the already low percentage of failures, and eliminating the potentiality of further enhancing the resistance of the organism under treatment. A further and final refinement in our armamentarium will be the determination and use of that fraction of penicillin that exhibits the greatest activity against the gonococcus.

A series of cases, in which the diagnosis was nonspecific urethritis, prostatitis, epididymitis, pyelonephritis, and infections such as wound infection and balanitis, has been improved by penicillin therapy, in 85% of the cases. Clinical evidence of response to treatment is quite definite, if penicillin is of value in the particular type of organism, and the response comes about with the use of comparatively small amounts of the drug.

TABLE 4

RESULTS WITH PENICILLIN THERAPY IN VENEREAL DISEASES*

Month 1945	*Cases Treated*				*Number Relapsed*			
	Gonococcus urethritis	Syphilis	Chancroid	Lymphogranuloma venereum	Gonococcus urethritis	Syphilis	Chancroid	Lymphogranuloma venereum
May	808	93	13	1	42	3	0	0
June	789	72	0	0	43	10	0	0
July	901	106	0	0	61	4	0	0
August	757	81	1	1	39	3	0	0
September	695	62	1	0	30	3	0	0
October	658	59	4	0	36	2	0	0
November	646	95	5	0	40	0	0	2
Total	5,254	568	24	2	291	25	0	2

* Compiled from reports of all activities, Eleventh Naval District.
Five per cent of gonococcus urethritis cases treated with penicillin showed relapse.
Four per cent of syphilis cases treated with penicillin showed relapse.
All cases responded on re-treatment.

Penicillin in Dermatology and Syphilology[1, 35, 36]

Of the 892 syphilis patients treated in this series, 32 showed evidence of clinical or serologic relapse.

The present routine for treatment can be summarized:

1. Early syphilis: 40,000 units, every 3 hours, for 60 doses; a total of 2,400,000 units, during a period of 7½ days.
2. Latent syphilis: 40,000 units, every 3 hours, for 100 doses; or a total of 4,000,000 units, in 12½ days.
3. Central nervous system syphilis: 40,000 units, every 3 hours, for 200 doses; a total of 8,000,000 units, in 25 days. This is concluded with 10 units of artificial fever therapy, accompanied by 10 intravenous injections of 60,000 units each.

In cases of uncomplicated primary sores, it was extremely gratifying to note the disintegration and disappearance of the *Spirocheta pallida* from the local lesion, in from 7½ to 10 hours, and complete healing of the lesion, in from 3 to 15 days, depending on its size and/or a complicating phimosis. Reactions were encountered, in 10% of the cases, consisting of rise in temperature, urticaria, generalized pruritus, and Herxheimer reactions (50%). Fever ranged up to 105° F. and, interestingly enough, usually reached its peak after the third injection, when most of the spirochetes had disintegrated and disappeared from the local lesion.

Penicillin is certainly the best drug ever made available for the treatment of syphilis, when judged on the basis of safety, better toleration, rapid sterilization of the infection, with disappearance of the treponema from the local lesion, and reversal of both blood and spinal fluid serologic findings. From the organized, nationwide, governmentally-sponsored studies, from which definitive results may be expected rapidly to emerge, will come the answer of the optimum methods of its use; whether alone, or in combination with other forms of treatment.

About 400 patients with various skin conditions have been treated with penicillin, locally or systemically. The pyodermas, including impetigo and sycosis vulgaris, responded nicely to the drug, employed either systemically or locally. Furunculosis was controlled, temporarily, by systemic treatment, while the following were not affected: acne vulgaris (except the secondary pustular elements), erythema multiforme, mycosis fungoides, dermatitis herpetiformis, eczema, fungus infections, and scabies. In cases of acne associated with cyst formation, gratifying results in involution of the cysts, without the usual disfiguring scarring, has been achieved by draining the cystic cavities, followed by the instillation of penicillin and infiltration around the cyst. It has also been my observation that, in certain types of vesicular and bullous infections of the hands and feet, complete involution, with exfoliation of the epidermis, followed by rapid healing, occurs with entirely non-specific therapy. This suggests the possibility of development *in situ* of antibiotic substances by bacteria, and is worthy of further investigation.

Phagadenic ulceration, which occurs commonly among the natives in most tropical countries, and which ranks second only to malaria as a principal cause of death, responded to the local application of penicillin, in a series of 18 cases. In some cases, healing progressed so rapidly that skin grafting was not necessary. Bacteriologic studies revealed the presence of spirochetes and fusiform bacilli, often in pure culture.

Penicillin was also found effective in 24 cases of chancroidal infection, although it failed to cure three experimental cases, possibly because of inadequate dosage.

Miscellaneous[1, 32, 37–41]

Penicillin was found to be ineffective, in tuberculosis, lymphosarcoma, carcinoma, infectious mononucleosis, chronic ulcerative colitis, leukemia, brain tumor, rheumatic fever, malaria, filariasis, mumps, coccidioidomycosis, and agranulocytosis.

One case of acute suppurative pericarditis was successfully treated with intrapericardial penicillin and systemic chemotherapy. In all, 100,000 units of penicillin, in 5 daily doses of 20,000 units each, were instilled into the pericardial sac, following pericardial paracenteses, combined with the systemic administration of sulfadiazine and 1,740,000 units of penicillin.

Another case can also be added to the growing list of successful management of subacute, bacterial endocarditis with penicillin. The drug was continued, in a dosage of 10,000 units, every 4 hours, for 7 days following remission of fever, and the treatment was further supplemented by supportive measures, including repeated transfusions of whole blood. But, as has been pointed out by other investigators, some patients have recurrences, as long as 6 to 9 months after treatment. It is, therefore, too early to pass final judgment, in this case.

SUMMARY AND CONCLUSIONS

1. Herewith is presented a study and review of the use of penicillin, either alone, or with conjunctive therapy, in sixty-five different clinical entities, aggregating 17,879 patients.

2. The source of this material, in addition to that of the author's, is entirely from the work and previously published and unpublished reports of physicians in the Naval Medical Service.

3. Problems of dosage are discussed, and more recent observations on the effectiveness of penicillin in influenza bacillus pneumonia and primary atypical pneumonia are presented. The restriction of fluids, beyond maintaining a urinary output of 1500 ccm. per day, is advocated for its definite value in maintaining higher blood levels and forestalling pulmonary edema.

4. Penicillin, administered systemically, diffuses into spinal fluid in adequate concentration, during the course of severe meningitis. However, since both penicillin and sulfadiazine are highly successful

in the treatment of meningococcal infections, no categorical decision can be made between the two. In order to conserve valuable time, and in the interest of saving lives, the initial concurrent use of both drugs is advocated, while awaiting completion of bacteriologic studies. Since pulmonary edema was a post-mortem finding, without exception, restriction of fluids would seem to be advisable.

5. In the specialties of otorhinolaryngology, ophthalmology, dermatology, and in the control of wound infections, penicillin has a definite place, both topically and systemically.

6. Penicillin is not a substitute for the surgeon's scalpel, in appendicitis or brain abscess, but is a valuable adjunct in counteracting intra-abdominal complications in the former, and promoting a lower mortality rate in the latter.

7. Yaws responds promptly to penicillin therapy, but final evaluation, in the treatment of this disease, awaits the test of time.

8. Although the results with penicillin, in gonorrhea and early syphilis, are better than with any drugs hitherto available, further and final refinements in the treatment of these diseases await the determination of that fraction of penicillin that exhibits the greatest activity against the gonococcus, and optimum methods of its use in syphilis, whether alone or in combination with other forms of treatment.

9. The observation of the rapid involution of vesicular and bullous infections of the hands and feet, without specific therapy, is worthy of further investigation, from the standpoint of the development *in situ* of antibiotic agents produced by bacteria.

10. In addition to those already mentioned, this study points out further problems awaiting solution, such as: the use of penicillin in prophylactic therapy; the value of high blood levels, in conditions thought to be unresponsive to penicillin; the slowing up of its absorption, and the further development of its oral use; the elimination of variability in potency of the drug, and the need for an expression of dosage in grams, rather than the present unit designation.

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